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EVALUATION OF RABBIT PRODUCTION AND WELFARE

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GENERAL SECTION

1. ANIMAL WELFARE

1.1 Notes on animal welfare in intensive farming

Public opinion is increasingly attentive to the conditions of animals kept in intensive farming and to their life quality. The considerable intensification of production systems that characterizes the livestock sector has caused an increase in welfare problems for the various farmed species (Verga et al., 1999).

In 1965, the committee for the study of animal health set up in Great Britain, better known as Brambell Committee, published the Brambell report, in which it focused on the lack of behavioural studies regarding animals housed and subjected to intensive farming systems, claiming that these studies are important for both animals and livestock production. The report also pointed out that any valid and serious estimation of animal welfare must be based not only on physical evidence of productivity, but also on the behaviour of farmed animals; it stated that it should be taken into account "the mental experiences of animals, their feelings and their impulses" and "that there is no reason to attribute a 'mind' to man and not to animals, since mental functions are of neurological origin in humans as well as in animals". It is interesting to note that the Brambell report proposed a sort of anthropomorphization, as parameter for evaluating and quantifying the suffering of an animal, arguing that the only way to properly measure the suffering of another being is to use the same parameters as for our suffering, such as vocalizations, expressions and reactions. The animals undoubtedly show signs of pain, fear, anger, frustration and other emotions (Verga and Carenzi, 1981).

Over the years various definitions of animal welfare have been proposed (Verga and Carenzi, 2009), starting from that of Hughes (1976), according to which "welfare is a state of perfect physical and mental integrity in which the animal is in complete harmony with the environment that surrounds it". This concept has undergone a series of evolutions up to the definition of Broom (1986), according to which "the welfare of an organism is its state in relation to the efforts it makes to adapt in an ideal way to the environment that surrounds it".

The most comprehensible definition and immediately applicable to farmed animals is that of the 5 freedoms, enunciated for the first time by the Brambell report in 1965 and then resumed by the Farm Animal Welfare Council (FAWC) in 1991, which states that animals must be free:

- from thirst, hunger and malnutrition;
- from inadequate housing;
- from pain, injuries and diseases;
- from fear and stress;
- to express the normal species behaviour

The 5 freedoms have been reviewed rather recently and modified as described below (FAWC, 2011):

- freedom from hunger and thirst with easy access to fresh water and a diet to maintain full health and vigor;
- freedom from discomfort by offering an appropriate environment that includes a shelter and a comfortable resting area;
- freedom from pain, injuries or diseases with prevention or rapid diagnosis and treatment;
- freedom from fear and anxiety ensuring conditions and treatments that avoid mental suffering;
- freedom to express normal behaviour by providing sufficient space, adequate equipment and company of conspecifics.

The first 3 freedoms are easily identifiable and measurable and, generally, pursued by the farmer for the strong consequences on productivity. Ensuring the absence of fear and the freedom to express the species-specific behavioural repertoire can be difficult, especially in the case of farmed rabbits (Trocino and Xiccato, 2006). To improve the welfare conditions of farmed animals, it is considered appropriate to use 2 strategies: on the one hand to promote productions that guarantee an adequate quality of life for the animals, therefore a good quality of the production process as a whole; on the other hand, it is necessary to introduce regulations that define the minimum conditions that allow to provide acceptable levels of welfare (Verga et al., 1999)

Welfare is closely linked to the health of domestic animals, in fact exposure to stressful activities can lead to the onset of diseases or to an increase in susceptibility towards them. Proper welfare management must include appropriate facilities for the species being farmed. It is necessary to provide a sufficient area, so that the animals can access the feeders without the onset of competitions; an adequate environment, therefore temperature, humidity, ventilation and air movement within the ranges required by the species; finally, comfort and freedom from fear imply that animals are housed in cages or structures adequately sized and suited to the biological and ethological needs of the species (Sainsbury, 1986).

1.2 Rabbit behaviour in wildlife condition

The rabbit differs from the other farmed species as it is the only domestic animal whose behaviour is assessed on the basis of the behaviour of the wild animal (Morisse, 1998). In fact, the process of domestication is rather recent and has not induced marked behavioural differences from the wild rabbit, if not in the entity and frequency of some behaviours, such as for example the greater diurnal activity of the domestic rabbit. The biological characteristics of rabbits are described in the introductory chapter of the Drafts of Recommendations on rabbit welfare in intensive farming of the Standing Committee. In nature, rabbits prefer grassy areas with soft soil, to be able to easily dig the burrows in which they live, and with a thick undergrowth, to be able to hide easily in the presence of a predator. They are herbivorous animals that are distinguished by the cecotrophy behaviour and, in semi-natural conditions, from 30% to 70% of the day, is spent in the search and ingestion of food, depending on the season. They are gregarious animals, which spend most of their resting time in groups and in close contact, showing a marked social activity which, in effect, is not feasible under the typical farming conditions (single or bicellular cages) (Trocino and Xiccato,2006).

The social unit consists of an average of 1-9 females, 1-4 bucks and in the colony agonistic interactions are infrequent because hierarchies are well defined and controlled by the emission of pheromones. The adult males show tolerance towards the females and the young rabbits, while a certain competition can sometimes be among the females for the choice of the nest construction site (Mc Bride, 2004).

While in nature the rabbit has a mating period that depends largely on the season, and therefore mates especially from spring to autumn, whereas under farming conditions the environment is usually kept constant, so the only unsuitable period for mating is the moulting period (Verga and Carenzi, 1981).

On farm, the conventional reproductive rhythm is based on artificial insemination (AI) 11-12 days *postpartum* (*pp*), rather than mating immediately after the kindling in order to avoid excessive exploitation of the rabbit doe. Reproductive performances are kept high throughout the year by constant photoperiod modulation at 14-16 hours of light, while the use of artificial insemination does not allow the expression of the precopulatory and mating behaviours typical of wild rabbit and still present in domestic ones (Trocino and Xiccato, 2006).

While in nature the heat does not appear outside the mating season and the female, although relatively receptive to the male, does not become pregnant, in rearing systems the doe is suitably synchronized and treated to be pregnant. The presence of oestrus is generally characterized by a red/purple and turgid vagina and by a certain restlessness of the animal, which remains still when a pressure similar to that of the mount is exerted on it (Verga and Carenzi, 1981).

The rabbit doe does not have a spontaneous ovulation, but induced by mechanical or hormonal stimulation (Hafez, 1993), but even the environment can directly influence the sexual behaviour. Observations in rabbits show the importance of olfactory sexual communication: the partners emit pheromones of a particular odour, as a sign of attraction and recognition (Verga and Carenzi, 1981). In nature, seasonal changes were noted in the amount of odours emitted, which become much more evident and stronger during the mating season.

As described in the Recommendations, the maternal behaviour of the rabbit doe differs from that of other mammals, due to the minimal parental care provided towards the litter (Figure 1). Under wildlife conditions, the rabbit moves away from the common burrow 3-4 days before kindling and looks for a place to dig a new nest (Trocino and Xiccato, 2006). The nest is made of grassy material and, just before giving birth, with the hair plucked from their breast and chest. She gives birth to three to eight hairless and blind kits. Immediately after birth, the doe leaves the nest, closes it and only visits her kits once a day for suckling. Suckling occurs once a day, usually after sunset, for a few minutes (2-5 minutes). After suckling, kits dig themselves deep into the nest, resting in close contact with each other (Myers and Schneider, 1964). In the first two weeks of lactation, the kits begin to ingest the faecal pills of the mother and other solid material, thus initiating the cecal fermentative activity (Kovács et al., 2006; Combes et al., 2014).

The nest is opened by the doe 18-20 days after the birth. In the case, frequent in nature, in which the doe has been fertilized immediately after kindling, milk production decreases drastically from the 20th day of lactation onwards and, around 24th-25th, she definitively leaves the nest and the litter to get ready for the next kindling. If not pregnant, the weaning of the litter is completed between 4th and 5th week of age (Trocino and Xiccato, 2006).

Figure 1. Litter of wild rabbit.

It is interesting to note that rabbit does usually do not manifest the so-called "retrieving", that is the recovery of the kit, and it seems that this is the case since it hardly manages to escape from the nest and is generally oriented towards it with the snout, therefore potentially to return slowly by itself (Verga and Carenzi, 2009).

Recommendations describe also other characteristics of the behavioural repertoire of rabbit, such as the various comfort activities aimed at both their own body (e.g. grooming) (Figure 2) and other animals (e.g. allogrooming), and locomotor activities. The latter are very particular and include the jump as the main expression (Figure 2).

Figure 2. Grooming and jumping activities.

Rabbits normally move with small hops and make longer jumps to overcome any obstacles or reach high positions. The housing systems currently used in intensive farming limit the possibility of movement and preclude the most typical locomotor activities. The exploration activity of the rabbit is evidenced mainly in digging and sniffing the surrounding environment, which is often associated with masticatory activity. Anti-predatory responses include alert positions (Figure 3), rapid escape to shelter or immobility. In the latter case, it is called “freezing”, by which the rabbit tries to escape the aggressor. In nature, it is also possible to observe a rabbit guarding the common burrow (Figure 3) that communicates to the companions the presence of a danger, beating the hind leg on the ground. In commercial farms this behaviour was not observed in growing rabbits, whereas it was sometimes described in adult ones (Trocino and Xiccato, 2006).

Figure 3. Alert position and defence of the burrow.

Regarding social life, the rabbit has an organized life both in terms of time and social structure (Mc Bride, 2004). A rabbit is a creature of habit: it emerges from the burrow at dusk to return to it after dawn. A large territory can provide space for 50 or more rabbits. This territory can be imagined as a village whose total population is called "colony". The colony includes several smaller and distinct social groups among them. A group can also consist of only 2 animals, usually a male and a female. The groups rarely have more than 8 members and the females are usually more numerous than the males. Females of a group tend to be closely related. Each group has a social organization that aims to maintain harmony among its members. Among the males, the hierarchy is quite rigid, with the dominant male, often larger and older than the others, which has priority over females for mating and the best places to feed and rest.

These so-called dominance hierarchies are not maintained by the dominant rabbit using aggression or by being a bully (Mc Bride, 2004): the other rabbits evaluate their chances of winning a possible contest and, if the comparison does not seem to hold up, they show a submissive behaviour and give precedence to the most dominant animal. When young males mature, they challenge the dominant rabbit; for this reason, they are usually pushed out of the group before they reach full maturity, reaching new groups in the colony or even leaving the whole territory and look for a new one. They tend to be accepted in a new territory when the mating season is over for that year: in this way the colonies avoid crossing. In the period of transition in which they are sent away from their original group and find a new territory, they tend to live on the surface, resting in shallow depressions or hidden under the bushes. During this period of wandering and life on the surface they are called "satellite" rabbits. Other satellite males are often old animals that have lost their authoritarian position in the group and have been ousted by younger and stronger males (Mc Bride, 2004).

Even among the females there is a hierarchy, often with only one dominant female, but this social structure is much less rigid than the male one and the dominant females are more tolerant towards the other females of the group. However, during the reproductive season, they can become extremely aggressive towards the same rabbits with whom they spent autumn and winter. The most important thing for does is to find a dry and safe place to build the nest, but these places may not be so available and there may be cases of females fighting to the death to establish the right to take over a particular site to give birth.

Another very important aspect of a rabbit's life are their senses. They spend most of their time in darkness and therefore do not tend to use visual signs as the main channel of communication. The main mean through which they communicate their position, sexual status and health is smell (Mc Bride, 2004). In fact, rabbits are almost completely mute, a precaution to avoid attracting predators. Communicating using sounds would give a predator the opportunity to localize them. The rabbit’s vocal repertoire is therefore very limited compared to that of other species.

Vocalizations tend to denote pleasure, pain or aggression that can be motivated by anger or fear. Like cats, rabbits make a purring sound when they are happy. They also produce a slight popping noise and when you hear a slight creaking of the teeth it seems to mean that the rabbit is relaxed and satisfied. A rabbit that does not want to be approached clarifies its position by grinding its teeth or making a series of grunts and growls. The most surprising sound emitted by the rabbit is the shout: the animals shout only *in extremis* when they are very scared or attacked by a predator. This behaviour represents a desperate attempt to scare the predator and a warning for every other rabbit nearby. Even kits that are still in the nest make noises to try to scare predators.

A litter, if disturbed, even if they are blind and deaf kits, starts to jump up and down in a synchronized way: certainly, it is unlikely that this strategy will work with a large and expert animal, but it could be enough for a small and young one.

1.3 Behaviour and welfare of rabbit in intensive farming system

Due to the lack of information on welfare of rabbits in intensive farming, in 2005 the European Food Safety Authority (EFSA), on behalf of the European Commission, drafted a summary document of all scientific data available on rabbit farmed welfare (EFSA, 2005), concluding that current intensive farming systems do not respect some of the behavioral peculiarities of the animal.

The study of behaviour is fundamental to understand the needs of the species and consequently the adaptation to the farming conditions but, among the various indicators, the behavioural ones are often difficult to evaluate and interpret due to the tendency of considering them separately from the context (farm). In the case of rabbits, the evaluation of behaviour in intensive farming is even more complicated than in other species, since it is a recently domesticated animal, for which the behaviour of the animal in nature is often referred to (in very different conditions from those of confined breeding) to evaluate the possibility of the animal to express its normal behavioural repertoire.

Although there is not yet an animal model referred to for the farmed rabbit, the observation of behaviour is used to compare different housing situations and to identify the appearance of anomalous behaviours. On the other hand, reactivity tests can be used to assess the rabbit's response to new environmental situations and its condition of stress and fear, with a series of difficulties in interpreting the response of animals (Erhard and Mendl, 1999).

Animal welfare problems related to the impossibility of rabbits to express normal behavioural patterns under intensive farming conditions are different depending on whether we consider the reproduction sector or the fattening sector. Regarding reproducing rabbit does on commercial farms, these are housed individually in cages that are able to host the mother and the litter until weaning. Since rabbits express gregarious social behaviour in nature, individual housing is critically considered by animal welfare advocates. Life in a limited space does not allow the doe to display natural behaviours and social interactions, but it can also lead to different abnormalities of the skeleton resulting from unnatural postures (Dresher, 1996). In effect, the current dimensions of the cages (65-70 of depth x 30-35 cm of height) limit the possibility of movement of rabbit does and do not allow them to run, jump and rise on their hind legs with erect ears. Furthermore, the environment is often bare and can induce abnormal behaviours such as stereotypes, aggressions and/or apathy (Morton et al., 1993). Among stereotypes, the repeated biting of cage bars has often been observed. To allow animals to fully relax and turn around, the depth and height of the cage should be at least 75-80 cm and 40-50 cm, respectively (EFSA, 2005).

The group housing of does allows social contacts and the expression of natural behaviours (Mugnai et al., 2009). However, the management of animals under reproductive activity kept in a group with the contemporaneously present litter is very difficult and not economically sustainable. Females can show aggressions towards each other and towards the kits of the other litters. Such negative behaviours can be contained housing females in groups already before the reproductive phase, to make them accustomed to conspecifics and to form stable groups (Luzi et al., 2009). It is a fact, however, that even in nature the activities related to the care of the offspring are extremely limited in the rabbit and are not shared in any way with the other members of the colony.

The EFSA in 2005 stated that insufficient knowledge and technology was available at that time to recommend implementation on farms of the group-housing of reproducing does. Indeed, recently literature confirmed that continuous group housing usually results in very high rates of aggression among females and competition for nesting areas, which impairs animal welfare in terms of frequency and degree of injuries among reproducing does, as well as towards kits (Andrist et al., 2013; Szendro et al., 2013). Moreover, even in 'part-time' housing systems, in which reproducing does are kept in a group during some periods and individually in others, aggression, fightings and presence of injured rabbits (46–66%) after each re-grouping remain unsolved problems, as shown by several studies (Andrist et al., 2012, 2014; Rommers et al., 2014a; Buijs et al., 2015; Machado et al., 2016).

Different strategies (platforms, hiding place, group stability, regrouped into familiar or unfamiliar pens, sprayed odours) have been tested without huge success to reduce aggression at regrouping (Graf et al., 2011; Rommers et al., 2013, 2014; Buijs and Tuyttens, 2015). According to Zomeno et al., 2018 the time of group formation (first days after kindling, early or late lactation) may affect the aggression level among does, being females in late lactation may be less stressed, since more time has passed after kindling and kits are less vulnerable.

Regarding growing rabbits, group housing of 5-8 animals is a common practice in the rest of Europe (especially France and Spain), while in Italy the bicellular cage is more often used. Italian producers justify this choice with the higher weight required by consumers (2.5-2.7 kg on average) compared to other European countries and the consequent greater age of slaughter (80-90 days) which makes aggressive behaviours and animal injuries more frequent (Trocino and Xiccato, 2006; Xiccato and Trocino, 2007). The most common bicellular cages in Italy have a surface of 1200 cm^2 corresponding to a farming density of 16-17 rabbits/m^2. According to EFSA recommendations, fattening rabbits should be kept in collective cages with a depth of 75-80 cm, a width of 35-40 cm and a height of 38-40 cm. The minimum surface area available per rabbit should be 625 cm^2 with a maximum slaughtering load of 40 kg/m^2 as they show that they are in a critical situation at a density greater than 15-20 animals/m^2 or a load greater than 40 kg/m^2. Above these values, the availability of space for movement decreases and access to feeders is more difficult especially in the last weeks of the fattening period. While housing conditions in bicellular cages strongly limit the animal's locomotor possibilities (Figure 4), the multi-purpose cages, designed to first host the rabbit does with the litter and then the growing rabbits until slaughter, are more suitable for group housing (EFSA, 2005).

Figure 4. Rabbits in bicellular cages under intensive farming conditions (photo by Angela Trocino).

On the other hand, bicellular cages facilitate the identification and treatment of sick animals and make farm surveillance and monitoring easier, proving easier to manage from a hygienic-sanitary point of view (Verga et al., 2007).

However, if we consider the possibility of expressing a normal behaviour for the species, the bicellular cage is a very questionable solution not only for the limitation of space, mentioned above, but also for the limitation to the expression of social behaviours. Rabbit under wildlife conditions, as mentioned above, lives in small colonies of limited size in close contact with other subjects. In a bicellular cage, the rabbit is in close contact with only one conspecific, it has a limited space to be able to perform a natural movement (jump), there are also no objects to interact with, the floor prevents digging and this type of housing does not allow normal social behaviours. The group to be kept in a collective cage must be formed at an early age and an adequate number of animals is recommended, leaving together animals of the same litter, homogenous per body size (EFSA, 2005; Postollec et al., 2006).

When the fear of pet rabbits against humans was assessed, subjects raised in mono and bicellular cages were less fearful than those housed in colony. On the other hand, in the open field test, the evaluation of reactivity towards a new environment has shown a more passive behaviour, as an indication of greater fear for animals housed individually (longer time spent sitting) than those in colony (Schepers et al., 2009).

According to Morisse and Maurice (1997), the behaviour of young rabbits is little influenced by stocking density, while in the last period before slaughter (at about 10 weeks of age) the time spent resting increases with the density of housing and, at the same time, the time spent feeding and water intake decreases. Social interactions and locomotor activities are reduced and comfort activities abnormally increase above 15 animals (38 kg) per m^2. Based on these and other similar results, 40kg/

m^2 are considered the maximum acceptable slaughter load and also compatible with normal behaviour for the species (EFSA, 2005; Szendro, 2009).

The height of the current cages could also limit the expression of some behaviours, such as the alert position, that rabbits can take even in conditions of intensive farming. Examining several well-being indicators (preference tests, ear injuries associated with aggressive behaviour, performance), it can be concluded that: the most widespread cages (30-35 cm high) seems to be adequate for fattening rabbits (Princz et al., 2008), while they are a problem for rabbit does, considering the greater dimensions of the breeding animals and the longer productive career.

Also the type of flooring that constitutes the bottom of the cages can affect the behaviour of rabbits and its general condition of well-being. From a behavioural point of view, the wire mesh currently used for the bottom (as well as for the whole structure of bicellular or multi-purpose cages) does not allow some typical behaviours of the rabbit in nature, such as digging. On the other hand, the use of a full bottom is unfavorable from the point of view of health and hygiene due to the susceptibility of the animals to coccidia infestations and to the ease with which the animals could get dirty. The use of straw as litter would allow the rabbit to enrich its behavioural repertoire, but so far this material has turned out to be unwelcome to the animal that shows a clear preference for the straw-free area (Dal Bosco et al., 2002; Morisse et al., 1998). The type of floor can negatively affect the well-being if it is unsuitable for the animal, limiting the possibility of movement (e.g. slippery ground, presence of obstacles) or causing injury to the animals (e.g. nets with inadequate rods). In the fattening sector, these problems are considered in a limited way, since the animals are reared only for a limited period of time (10-13 weeks), not enough for the manifestation of health problems such as pododermatitis, which is frequent in the breeding animals, where the use of a plastic bottom with grids at least 1 cm wide or the placement of a slotted mat in the resting area above the metal mesh can limit the formation of sores both in bucks and rabbit does (Luzi et al., 2009; Conficoni et al., 2020).

Finally, microclimatic conditions of the farming environment are very important for optimizing production performances and for guaranteeing a general condition of comfort, limiting animal stress (Figure 5). The main factors to be controlled are temperature, relative humidity, lighting and air quality (harmful gases) (Luzi et al., 2009).

Figure 5. Overview of rabbit intensive farming (ww.consorziofinagro.it).

Farming methods for rabbits can also influence the ability of animals to react to the stress linked to slaughter events in a more or less positive way and allow for a better control of the animals' fear in these phases. It is known, in fact, that events that occur before slaughter (food and water withdrawal, transport, lairage) are critical for animal welfare (Verga et al., 2009). Before transport, rabbits are removed from the cages where they are grown and hand-loaded into the transport cages. The European Union has issued a specific regulation on the protection of animals during transport (EC, 1/2005), in which the provisions relating to rabbits are similar to those relating to poultry, with indications on the shape and size of the cages that must allow the animals to be in a natural position, provide adequate ventilation and an inside temperature between 10 and 20 ° C. Finally, the duration of the transport must not exceed 8-12 hours. More recently, EFSA (2011) has produced an updated document on animal welfare during transport (Scientific Opinion Concerning the Welfare of Animals during Transport), on the basis of which it has provided some specific recommendations, indicating the level of risk related to various aspects of transport (e.g. means of transport, transport practices and space requirements), as well as a series of practical indicators and clinical measures that employees can use to assess the welfare status of animals. The document dedicates a specific section to rabbits and concludes that temperature-related stress during transport can harm welfare and that it is appropriate to ensure adequate ventilation during transport to maintain the internal temperature in the optimal range. Furthermore, more scientific information on the effects of temperature on rabbits during the transport phase to the slaughterhouse is required.

1.4 Relationships between stress, behaviour and state of health

Animals react to difficult situations by altering their behaviour first. Behavioural responses are often correlated with both physiological and immune aspects. Consequently, behavioural stress indicators can be used to predict the effect of the latter on the biological functions of the animal. There are several reasons why an animal on a farm can be found in conditions of discomfort such as to cause fear and anxiety, which can undermine the condition of well-being and which can lead to changes in behaviour.

Fear and anxiety are two closely related emotions. Fear is usually defined as the reaction to the perception of real danger, while anxiety is the reaction to the potential danger that threatens the integrity of the individual. The reactions related to fear are characterized by physiological and behavioural responses that prepare the animal to face danger. Routine management procedures can elicit responses related to fear e.g. shearing, castration, cutting of the tail, vaccination, transport in cows, pigs and sheeps. In addition, excessive fear could lead to the development of chronic stress with alterations in fundamental behaviours (social, sexual and parental relationships) and negative consequences on productivity (Forkman et al., 2007).

Social isolation is probably one of the most important stress factors for all social species. Many studies suggest that most domesticated species have a high social motivation and consequently would suffer more from separation anxiety than from the manifestation of fear aroused by itself. Many of the tests used in domestic animals to measure fear have previously been designed for laboratory animals: in domestic animals, young are generally early and the social dimension is extremely developed in most species that are very sociable and have an exclusive mother-son relationship, therefore testing animals in an unfamiliar environment can lead to an inaccurate estimate of fear (Forkman et al., 2007).

Lack of familiarity and unpredictability produce reactions of fear in the animal. For example, sudden and unknown events are often used in various species to assess the degree of fear of the animal, using reactivity tests that will be described later. From an ethological point of view, suddenness, lack of familiarity and unpredictability are the main features of a predatory attack.

On the other hand, if the animal is subjected to prolonged stress, there is a modification of the physiological scenario with a negative effect on the immune system which leads to a decrease in defenses and a consequent greater incidence of pathologies.

The activation of the hypothalamic-pituitary-adrenal axis is one of the most known neuroendocrine adaptive stress responses. Psychological, environmental and physiological stressors can cause the release of the corticotropin releasing factor from the anterior portion of the hypothalamus, which stimulates the secretion of the adrenocorticotropic hormone (ACTH) by the anterior pituitary gland. The berth organ of ACTH is the adrenal cortex and the main function of this hormone is to raise the secretion of corticosteroids (cortisol and/or corticosterone) which play an important role in coping with a stressful situation. Corticosteroids play a role in many regulatory functions of the body and their increase causes protein catabolism, hyperglycemia, immunosuppression, increased susceptibility to disease and depression. As a consequence, they could improve the immune function at one concentration and inhibit the same functions at another (Sevi, 2009).

The model of hormone-behaviour interaction (Figure 6) incorporates the notion that in many cases the success of adaptation depends on the opportunity for the animal to express an appropriate behavioural response. If animals are exposed to a cold current they change position and seek shelter, however if they cannot move away, they must increase their heat production or try to reduce energy expenditure in order to adapt to the air flow. The responses, which are initiated by the central nervous system, depend on the “brain state” at the time of stimulus perception. The behavioural response is given a specific quality aiming at the control of the threatening stimulus and a general quality which corresponds to two main modes of responding, either active (e.g. fight or flight) or passive (e.g. withdrawal, immobility or freezing). Peripherally released hormones depend on the behavioural attitude and they feed back to the brain to modulate the acquisition and retention of the behavioural response. In other words, the basic adaptive response is behavioural and neuroendocrine changes depend on the behavioural strategy adopted by the subject (Dantzer et al., 1983).

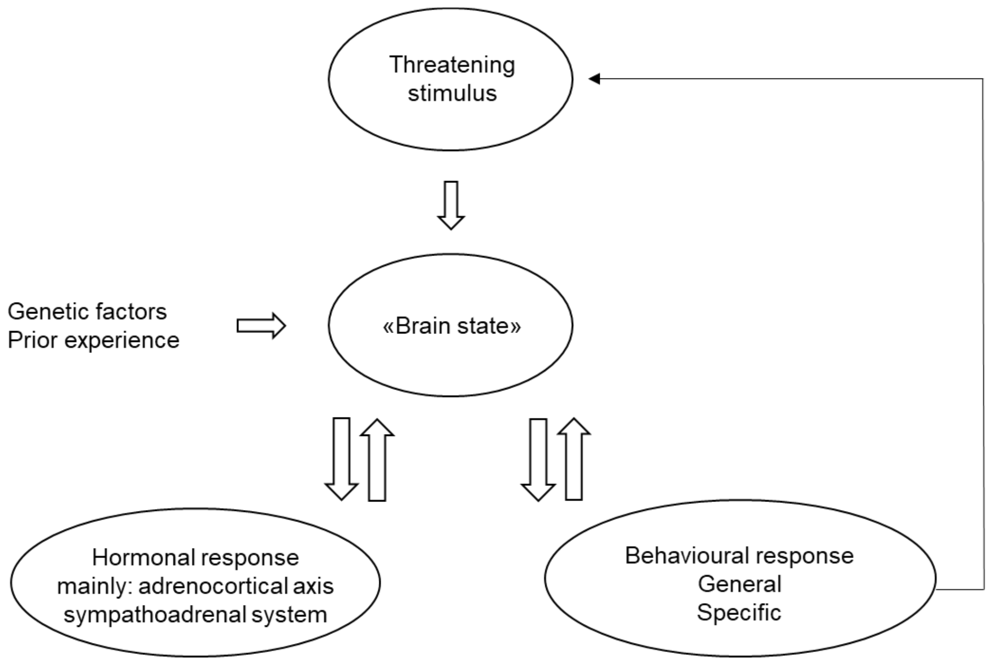


Figure 6. Model of the relationship between environmental threatening stimuli and bodily responses.

In addition to the hypothalamic-pituitary-adrenal system mentioned earlier, there is another fundamental neuroendocrine mechanism that takes part in the adaptive process and is the sympathetic adrenal medullary system, which is activated when the accessibility to the desired objects (e.g. food, water, shelter, partner) is limited and repeated attempts must be made to maintain control and possession. This response is associated with continued excitation and increased heart rate, blood pressure and peripheral resistance. On the contrary, the opposite situation, that is the deactivation of the system, represents not only the absence of the effect but also relaxation accompanied, for example, by grooming and affective behaviour (Sainsbury, 1986).

Both physiological and psychological stressors can cause the release of chitochines, which can cause increased production of neutrophils, can affect lymphocytes and capillary permeability, induce fever and an acute phase of protein synthesis. Furthermore, they act at the level of the brain, causing sleep and diminishing social, aggressive and exploratory behaviours, along with a reduction in appetite.

Regarding immunological responses, stress-induced corticosteroid secretion causes leukocytosis up to a few hours after stimulation and causes neutrophils to increase compared to lymphocytes. Also the humoral immune response, measured in terms of specific IgG antigen, can give an indication of the response to physical and emotional problems.

1.5 Evaluation of animal welfare in intensive farming

Given the above considerations, the variety of mechanisms activated to deal with stress requires a multidisciplinary approach and the use of multiple parameters for the evaluation of animal welfare under farming conditions. A very wide range of indicators is required because not all stress response mechanisms are always or simultaneously activated (Sevi, 2009). In the different farmed species, including the rabbit (Luzi et al., 2009), welfare can be evaluated using four types of indicators: productive, pathological, behavioural and physiological (Curtis, 1987; Broom, 1988; Ingvartsen and Andersen, 1993; Stull and McDonough, 1994; Grasso et al., 1999).

Productive indicators

They evaluate productive and reproductive performances (e.g. conversion index, weight gain, fertility, litter size at birth and at weaning). Among the different types of indicators, these are the most easily measurable. Productive indicators must be evaluated jointly with others, since if it is true that a worsening of reproductive and productive performances may indicate a worsening of animal's well-being conditions, on the other hand very high production performances are not necessarily linked with farming situations that respect animal welfare.

Pathological indicators

They indicate pathologies that are manifested or obvious. The presence of inadequate sanitary conditions is the cause of poor welfare on the farm and prolonged stress can lead to greater susceptibility to diseases due to the reduction of the immune response. In dairy cows, for example, the state of health can be assessed by checking the body condition using the Body Condition Score (BCS), verifying the condition of the skin and coat and detecting lameness and wounds; dirt can predispose animals to wounds and lameness, indicating an inadequate management (Sevi, 2009).

Behavioural indicators

It involves the analysis of the animals' behaviour, the identification of abnormalities and the evaluation of reaction to different stimuli or situations. The interpretation of behavioural indicators is always very difficult due to the complexity of the motivational system. In rabbits, it is complicated by the fact that there is no reference model on the farm. The rabbit, in fact, is an animal of rather recent domestication compared to other farmed species and for its behaviour evaluation we refer to that of the wild rabbit (Trocino and Xiccato, 2006). In behavioural analysis, the presence of stereotypes is an important indicator for poor animal welfare. They are defined as repetitive behaviours without a specific objective or function and can be triggered by a wide range of events. Distinguishing between stereotypes and other forms of behaviour can be problematic because: 1) there is often a continuum between the stereotypes and the normal behaviour from which they develop, 2) long-term stereotypes can become so habitual that the animal can incorporate them into normal behaviours. Most stereotypes do not appear ex novo, but they develop, so there is a continuum between the proto-stereotypes and the more developed ones. Stereotypes can be very different: some are induced by the environment and others by pharmacological and surgical interference with the nervous system; they can be invariable and repetitive to a very different extent and these two characteristics can be independent of each other (Lawrence and Rushen, 1993). The shape and persistence of stereotypes are often determined by the age of the subject. For example, the cage effect is increasingly marked on a young animal compared to an adult. Stereotypes that develop in the young are very difficult to delete and sometimes persist even when the animal is placed in an appropriate environment. In contrast, adult animals develop abnormal behaviours that can usually be altered or eliminated with the change of environment. In young animals they can be more serious because their central nervous systems are more plastic and therefore more profoundly altered by the environment.

Useful information on rabbit welfare can also be obtained through the so-called “reactivity tests” in which the reaction of animals towards a novel environment or man is measured. Reactivity tests are used to assess fear in farm animals, as widely illustrated by Forkman et al. (2007). The experimental design used varies with the nature of the fear to be tested, the environmental situation and the species considered. The results and responses of animals are often difficult to interpret and the tests developed are not always sufficiently validated (in terms of accuracy, specificity and scientific validity) and tested for reliability (Forkman et al., 2007).

Among the most widely used reactivity tests, the open field test measures the animal's reaction towards an unknown environment and is used in different species. In the specific case of the rabbit (Figure 7), it consists in inserting the animal inside a wooden fence divided into a certain number of numbered boxes and observing its behaviour during 5-15 minutes (Meijsser et al., 1989; Ferrante et al., 1992; Trocino et al., 2004; Verwer et al., 2009).

Figure 7. Open field test (photo by Trocino et al., 2013)

A high exploratory and locomotor activity during the test indicates a positive reaction and a low level of fear of the animal. On the contrary, a passive behavioural response, characterized by high alert, is considered passive and negatively evaluated because it indicates animal fear (Ferrante et al., 1992; Forkman et al., 2007, Buijs and Tuyttens, 2015). The response of the animals to the open field test can be strongly conditioned not only by the real fear of a new environment, but also by social deprivation that occurs when animals, kept in groups under farming conditions, face the test individually (Forkman et al., 2007). In the different experimental designs used for open field tests, even in other, a "suddenness" element can be inserted, using the presentation of a stimulus (e.g. ball suddenly dropped from the ceiling in front of the animal or a stroke of compressed air applied to the nose).

Among other tests, the tonic immobility test (Figure 8), also used in rabbits, evaluates the animal's fear towards man. The rabbit is taken by holding it behind the head and with a quick maneuver it is brought into a prone position thus entering a state of immobility, which reflects the behaviour in nature where it pretends to be dead to escape predators. During the test, the animal enters a pseudo-catatonic state and the duration of immobility is directly proportional to the fear it has towards the operator. Tonic immobility, especially in birds, is considered an effective indicator of the level of fear (Forkman et al., 2007). It indicates a strategy of active or passive behaviour in an adverse situation. In pigs, Erhard and Mendl (1999) have suggested that susceptibility to the duration of immobility can be seen as an indicator of the type of response to fear shown in a difficult situation rather than of fear itself.

Figure 8. Tonic immobility test (photo by Chiara Munari).

A further type of test utilized for ethological purposes is the so-called “preference test” in which the animal can choose, for example, between two different types of housing offered (Matics et al., 2004; Orova et al., 2004). During the preference tests the animal is placed in front of a choice between two or more different situations and chooses the situation that it perceives as most pleasant. It is necessary, during these tests, to recognize that the choice of the subject could be influenced by external factors and previous experiences that could affect the test result. It is also necessary to check the intensity of the preference, evaluating the intensity of the work that the animal is willing to do to implement its preference.

Physiological indicators

Animal welfare assessments also include physiological and immunological blood analysis, often to complete behavioural observations. Evaluation of changes on the level of endocrine and immunological indicators requires capture, treatment, manipulation by farmers and injections for animals. These procedures can be stressful for the animals and their effects can sometimes hide the stress factors to be tested. Cortisol or corticosterone levels, immunological measurements, chitochine release and an acute protein phase are considered reliable indicators for the evaluation of well-being. Using a less invasive way, it is possible to estimate the concentration of cortisol in the plasma of cows and sheeps directly from the concentration of salivary cortisol (Sevi, 2009) or the concentration of corticosterone in the hair and faeces of rabbits and other mammals (Buijs et al., 2011).

1.6 Housing systems and relative issues

Regarding the housing system, farms can be divided into conventional farms (including standard cages, enriched cages and elevated pens) and niche systems (including floor pens, organic and outdoor systems) (EFSA, 2020):

Conventional rabbit farms

Rabbit does are housed individually with their litter. Both inseminated but not pregnant does and replacement does are housed in small cages for around 5–8 weeks before entering into the production management system.

In some farms, the female always remains in the same cage after the litter weaning, to give birth for the next litter, whereas weaned rabbits are moved into new enclosures for the fattening period. In other farms, at weaning, the doe is moved to a new cage, whereas the litter remains in the housing system where they were born until slaughtering (all-in all-out system, using the so called "dual purpose cages").

Regarding **Standard wire cages** (Figure 9), widely spread under different European countries, they are equipped for the rabbit doe and its litter with a removable plastic nest containing the litter in the front; the nest area is separated from the rest of the cage by a removable wall with a sliding door. The door of the nest can be closed for controlled lactation during the first 16 days after kindling. Then, the wall between the nest and the rest of the cage is removed to obtain a unique space in which the mother and the litter will remain together till the weaning.

According to recent findings of The European Food Safety Authority (2020), this type of housing has the worst overall impact score for both rabbit does and growing rabbits, whereas for kits it reached the second lowest. The restriction of movement, together with inability to perform the typical behavioural pattern of the species (e.g. gnaw) and resting problems represent the main consequences of rearing in this type of cage for all categories. Restriction of movement is scored high for both categories of animals and is mainly due to lack of enough space and lower cage height (35 cm). The inability to gnaw was identified as a problem for all animal categories and it could be solved easily by adding enrichment materials to standard cages (e.g. wooden sticks). Resting problems in standard cages is a result of the wire floor which is considered uncomfortable, even if partially covered by a foot pad. In addition, in growing rabbits the high stocking density constrain resting behaviour for growing rabbits kept in standard cages, and total space allowance may do so for rabbit does (a problem that is likely to increase at high temperatures, which increase the females motivation to adopt a prostrated lying posture), while in kits the main problem is associated to soiled or inappropriately built nests. Also inability to express social interactions and heat stress were suggested as main welfare consequences for does in standard cages, the last indicating that current ventilation systems could be insufficient to prevent too high environmental temperatures, with negative consequences also for kits survival. Hunger is also considered a main consequence for kits and can be prevented optimizing feeding systems.

Figure 9. Example of standard cage (photo by Chiara Munari).

More recently, in some countries an increasing number of farms have started to use **enriched cages**, the so-called 'welfare cage', i.e. larger cages equipped with elevated platforms and plastic foopads, and sometimes other internal enrichment objects. This cage is larger and higher than standard cages. It is used for individual housing of the rabbit doe from a few days before kindling until the end of lactation with its litter and then, after removal of some items, for housing of growing rabbits. It is made of wire mesh and it is equipped with a feeder and a nipple drinker. It always includes a platform with wire mesh or plastic slats flooring.

From results showed by EFSA (2020), enriched cages got the second highest overall welfare impact score for growing rabbits and intermediate welfare impact scores for rabbit does and kits. Restriction of movement for all categories of animals and skin injuries for growing rabbits and kits were considered as major welfare consequences in this housing system. Inability to express gnaw behaviour for growing rabbits, resting problems for does and growing rabbits, inability to perform social behaviour and heat stress for does and kits were also ranked in the top 5 welfare consequences for this system with relative lower welfare scores. It has to be underlined that the occurrence of resting problems was actually the lowest in this type of cage compared to all other housing systems.

Moreover, a few farms use alternative systems based on **elevated pens**, usually called "parks", in which does are normally kept individually but may be grouped for some periods (part-time group housing) by removing mobile wire walls between single modules of a pen. This group systems are still being further developed in terms of housing management (e.g. regrouping strategies). They are made of single modules that can be connected together to allow group housing of rabbits. Each single module is larger than enriched cages and it can be open-top (elevated pens can be also made from enriched cages by removing wire walls between single modules). The single module is used for individual housing of rabbit does from a few days before kindling until the end of lactation with its litter and then, after removal of some items, for group-housing of growing rabbits. Walls are made of wire mesh, whereas flooring could be made of wire mesh or plastic slats. The single module is equipped with feeders and nipple drinkers. It always includes a platform with wire mesh or plastic slatted flooring to get the rabbit does resting when the kits start to come out of the nest. A plastic footpad is used if plastic flooring is not available. Single modules could be joined also for part-time group housing of rabbit does, but is not currently yet widely implemented in commercial farms (Figure 10).

For rabbit does, elevated pens were assigned the second lowest cumulative welfare impact score while for both kits and growing rabbits it obtained the lowest score. The score for does was similar to the one for enriched cages and outdoor systems and only slightly higher than the one for organic systems. Regarding the top 5 welfare consequences, restriction of movement contributes by far most to the overall impact score, followed by the relatively less important consequences inability of gnawing, skin lesions, resting problems and inability to perform positive social behaviour. Skin disorders was the most important welfare consequence contributing to the overall welfare impact score for growing rabbits, while resting problems, gastroenteric disorders, inability to gnaw and fear were judged to be relatively less important.

If elevated pens are not used for group housing of does, reducing restriction of movement requires fundamental changes to the housing system in terms of the total space available and cages dimensions. However, a platform and suitable gnawing material can be provided in existing systems. Group housing of does is the most important reason for skin lesions in does due to aggressive interactions between them and a prolonged hunger in kits and neonatal disorders seems to be associated to an inadequate nesting behaviour and poor maternal care. If elevated pens are used for individual housing, the does are however prevented from performing social behaviours between weaning and the next kindling. If elevated pens are used for growing rabbits, management protocols to avoid introduction of pathogens, climate control to maintain air temperature and relative humidity, are measures to reduce the occurrence of skin disorders (EFSA, 2020).

Figure 10. Example of elevated pen for group housing of rabbit does (photo by Chiara Munari).

Niche systems

These systems are usually for individual housing of rabbit does with their litter and for group housing of growing rabbits. Moreover, in Switzerland, small farms exist that use indoor deep litter parks, **floor pens**, for group housing of both categories. A total of about 3,600 rabbit does are kept in this system in Switzerland in small farms (Ruchti et al., 2018). This system is a niche system that uses indoor deep litter parks with plastic platforms for group housing of does or growing rabbits; males may be also present. No standards are available but, as a rule, they are large open-top pens based on solid floors with litter (Figure 11).

According to EFSA (2020), floor pens were the housing system with the second highest overall welfare impact score for does after standard cages, the second highest welfare score for kits after outdoor systems and an overall welfare score for growing rabbits which was intermediate among the housing systems. This results for rabbit does derived from a higher score for hunger than in other systems, with heat stress, resting problems, reproductive disorders and skin injuries. In general, physical and health problems featured more highly than behavioural problems. Additionally, in this type of housing pododermatitis might be a problem for does, scored higher than in enriched cages, elevated pens and organic system, but lower than in standard cages and outdoor systems. Many of these problems may reflect the hygiene challenges of floor pens, with the presence of soiled bedding contributing to reproductive, gastroenteric and skin infections, as well as to hypothermia and impaired resting. For kits, this housing systems reached the highest score for hunger, with also problems of thirst and neonatal disorders.

Figure 11. Example of wooden floor pen for group housing of reproducing rabbit does in Switzerland (photo by Michèle Braconnier).

As regards **organic systems**, basic requirements according to EU Reg 2018/848 include access to pasture whenever conditions allow for it, group housing, access to a covered shelter including dark hiding place, an elevated platform and nesting material. Implementation of these requirements will come into force from 2021. No further common standards in EU for housing of organic rabbit does with litters or growing rabbits have been specifically set until now. Except for organic farming, there are no standards regarding size of housing for other niche production systems, and no data are available about stocking density in terms of animals reared/m^2 and kg final live weight/m^2. Nevertheless, these differ according to several factors, i.e. animal genetics, housing conditions (including building, equipment and structures in which animals are kept), management of reproduction, management of growing/rearing, feeding, and biosecurity measures. In the EFSA new scientific opinion of this year, rabbit does showed the lowest overall welfare impact score among all housing systems. Similarly, for the growing rabbit, this score has a low range compared to the other systems. Restriction of movement is the highest welfare consequence for does, followed by heat stress, reproductive disorders and resting problems, whereas regarding growing rabbits, the top 5 welfare consequences were resting problems, gastrointestinal disorders, heat stress and fear. For kits, the three most important welfare consequences are represented by hunger, heat and cold stress.

Other niche systems use different fixed (cages, hutches, paddocks) or movable housing systems (usually cages) which may give access to outdoor areas and pasture, referred as **outdoor** systems. No standards are available for outdoor rearing but, as a rule, animals have the possibility of accessing an outdoor area, which is not necessarily pasture. According to the scientific opinion of EFSA (2020), the welfare score was the third across the six for rabbit does, it reached the second position for growing rabbits, while for kits it resulted the worst of all systems. Heat stress was the highest scoring welfare consequence and among the top 5 for does; additionally, a common welfare problem that affected both rabbit does and growing rabbits was resting problems. The main issues related to this type of housing are climate conditions and the difficulty to implement biosecurity measures. Therefore, improving housing to provide better protection would be an important measure to reduce climatic impact and fear.

2. REPRODUCTIVE MANAGEMENT

2.1 Managing of the doe

Reproductive performances of the female are influenced by sexual receptivity, lactation stage and the order of birth. By birth order we mean the number of pregnancies carried out by the doe (Theau-Clément, 2007).

Sexual receptivity can be assessed by examining the behaviour of the female during the presence of the male, or by evaluating the color and turgidity of the vulva. The greater or lesser receptivity indicates the states of estrus or diestrus, which are closely correlated with the levels of fertility and prolificacy. Fertility is obviously greater in receptive females, as well as the rate of ovulation and the rate of pregnancies (Zotte and Paci, 2013). It has been observed that by inseminating the rabbits at both 4 and 11 days *pp*, receptive does produce more numerous litters. All this leads to a productivity 3 to 4 times higher than that achieved by inseminating non-receptive females. (Zotte and Paci, 2013).

Considering receptivity and lactation jointly, it can be said that the worst results are achieved in non-receptive females inseminated 4 days *pp*, as a consequence of a worsening of the ovulation and fertilization rates of the oocytes. Prolactin, in fact, hinders sperm progression, increases fetal mortality, inhibits follicular growth and expression of luteotropic hormone (LH) receptors (Cerolini et al., 2015).

Concerning the order of birth it is established that, even applying the technique of AI, nulliparous females are generally highly receptive with respect to primiparous, with greater fertility (> 70%). The reasons for this are to be found in the antagonism between lactation and reproduction, and in the conspicuous energy deficit that occurs in primiparous to cover the demand for milk production, the development of fetuses and the achievement of an adequate body weight. In fact, the most commonly used hybrids are put into production at about 18 weeks of age, when their weight corresponds to 80-85% of adult live weight. Considering that lactation and gestation are usually overlapped on farms, it is important to analyze the response to AI in lactating does (Theau-Clément, 2007). Primiparous inseminated during their first lactation are generally less receptive and fertile, but produce a larger litter than that of a nulliparous (Theau-Clément, 2007).

The rate of receptivity varies considerably between kindling and weaning (Theau-Clément, 2007). Around 90% of females accept mating on the day of kindling. Instead, a noticeable reduction in receptivity is observed on the 4th day *pp* and a progressive increase from the 11th day up to the maximum levels immediately after weaning (28-30 days *pp*) (Cerolini et al., 2015). Lactating does, especially if the litters are numerous, are less receptive than non-lactating ones. Fertility decreases by 12-20% during lactation because it reduces the rate of ovulation, despite treatment with synthetic GnRH (Cerolini et al., 2015).

It should also be noted that rabbits inseminated 11 days *pp* are much more fertile than those inseminated at 3-4 days. The depressive effect on fertility is due both to the reduced ability to ovulate (51 *vs*. 79%, for 4 and 11 days *pp*, respectively), and to the lower number of pregnancies carried out (12 *vs*. 22% for 4 and 11 days *pp*, respectively), consequently to the failure fertilization or total mortality of embryos. Also with regard to fertility, the best results have been highlighted with increasing distance from kindling: the embryonic mortality one week after AI is very high in rabbit does inseminated at 11 days (14.5%) compared to non-lactating ones (4.8%) (Cerolini et al., 2015).

2.2 Body condition

Thanks to the knowledge obtained in rabbit farming, it has been possible to attribute a fundamental role to the body condition of the animals, considering it responsible for their reproductive efficiency, especially in terms of fertility and prolificacy (Naturil-Alfonso et al., 2017).
Several non-invasive methods have been validated to assess body conditions and the energy needs of rabbits; among these the most used technique is the Body Condition Score (BCS), a widely used subjective method that indicates the fattening state of the rabbit and its body condition, through the evaluation of specific anatomical regions (Menchetti et al., 2015).

Knowing how to manage young rabbits optimally means preparing them in terms of skeletal development, degree of maturity and therefore improving the ingestion capacity and the efficiency of utilization of ingested energy. The threshold value of the first AI is indicated as being a weight close to 4 kg, considering the lighter females not yet mature for the start of the reproductive career (Cerolini et al., 2015). Low energy intake can affect different aspects of pregnancy and lactation, resulting in delayed implantation, reduced fetal growth, pre-term abortion, birth defects, low birth weight and decrease in milk supply (Menchetti et al., 2015). This is explained by the fact that the weight at the first AI is strongly correlated to the number of kits and milk production (Cerolini et al., 2015).

In European intensive farms, the most common reproductive rhythm is based on the 11-day AI *pp* (semi-intensive), with the weaning of kits at 28-30 days of age. This protocol, however, does not take into account the physiology of the doe, so much so that often it is necessary to resort to the oestrus synchronization by hormones or biostimulation. On the other hand, it is known that by inseminating the rabbits after kindling (intensive rhythm), that is when the receptivity is maximum, the annual number of kindling/doe increases but the fertility, the numerical consistency of the litter and the duration of the reproductive career are strongly reduced. With the extensive rhythm, instead, (AI after weaning), an improvement in the body condition and an increase in their career is obtained. The risk of a possible fattening of this last group of rabbits should not be underestimated. The cycling, in fact, provides for the AI homogeneous groups of animals on a predetermined day, without taking into account the state of receptivity of the same. This means that often the females, found empty at the first AI, are re-inseminated after 3 weeks together with the others to make them return to the next group. The disadvantage is that the prolongation of the fertilization-conception interval could cause an excessive increase in weight (Cerolini et al., 2015).

Under normal conditions, the normal functioning of the ovaries, and therefore of ovulation, is conditioned by the hypothalamus and pituitary gland, which in turn are affected by the production of so-called steroid hormones, such as androstenedione and estrone. Androstenedione is produced by reserve adipose tissue; when the animal is overweight, androstenedione can give rise to estrone continuously. The more the doe gets fat, the more the amount of androstenedione increases, the more there is an excess of circulating estrogens, up to the point of disrupting the reproductive function. Obesity is therefore a widespread cause of infertility. Furthermore, fat deposits in the ovaries can interfere with embryonic development and cause spontaneous abortions (Lazzaroni et al., 2009).

In support of what has been said, the assessment of body condition is necessary, not only because it is related to the duration of reproductive efficiency, but also for the health and welfare of the animals (Menchetti et al., 2015).

2.3 Food needs and energy deficit

The feeding of young rabbit does needs to be followed carefully to allow them to reach the first insemination, pregnancy and lactation, in suitable body conditions to support the high nutritional requirements. The energy deficit leads to the mobilization of the body reserves and to the worsening of reproductive performance. As a result, the energy balance and life span are closely linked (Fortun-Lamothe, 2006).

As already indicated, females are generally inseminated for the first time at 16-18 weeks of age or when they reach 75-80% of the adult weight, that is 3.3-3.5 kg compared to 4.2-4.4 kg of adult subjects (Fortun-Lamothe, 2006).

In the first growth period, which goes from weaning (4-5 weeks of age) to puberty (10-12 weeks), the doe is fed *ad libitum* with moderately energetic feed for weaning and/or fattening and daily intake ranges from 100 to 170 g. Food is characterized by a low energy level and a good content of proteins and crude fiber (15.5-16% and 17-18%, respectively), to reach an adequate capacity of ingestion in the subsequent reproductive career (Fortun-Lamothe, 2006).

In the second period, which goes from puberty to the first insemination, feeding must be controlled to prevent fattening, the main cause of kindling problems or worsening reproductive performances (Naturil- Alfonso et al., 2017).

On the contrary, young females subjected to food rationing, at the time of the first AI often show a reduced sexual receptivity. This problem can be solved with an intensive food treatment called flushing, which consists in the administration of a lactation diet *ad libitum* for 4-7 days prior to the first AI (Cerolini et al., 2015). An *ad libitum* diet allows better sexual development, in terms of receptivity, ovulation rate, blastocyst size and implantation rate (Naturil- Alfonso et al., 2017).

Rabbit does feed contains high concentrations of digestible energy, highly digestible protein and biological value, as well as important levels of lysine and methionine, calcium, micro-minerals and vitamins. To cope with the particular nutritional requirements and the increased susceptibility to digestive diseases of the litter, the administration of the lactation feed generally begins a few days before the kindling and is interrupted 20-25 days after it (Cerolini et al., 2015). Afterwards, doe and litter are fed with a weaning feed, poor in energy but rich in fiber. This technique can favor the onset of a body energy deficit, which occurs when food intake is insufficient to cover the needs between one kindling and the next one. In fact, the capacity of feed voluntary ingestion is greatly reduced near kindling. As a result, the doe is forced to mobilize its own body reserves, especially the lipid reserves. The physical size of the feed in the digestive tract contributes to increase even more this deficit, because it prevents the intake of a sufficient amount of feed (Cerolini et al., 2015).

The negative energy balance is more serious in primiparous rabbits, which have a reduced capacity of ingestion and an incomplete somatic development, as well as in the multiparous rabbits up to the third-fourth kindling if subjected to intensive reproductive rhythms. As the kindling order increases, milk production increases, but to a lesser extent than the increase in energy intake and, therefore, the loss of energy from one kindling to the next is reduced to the point of being canceled in the rabbits from the fourth birth onwards (Cerolini et al., 2015).

An excessively thin rabbit doe recovers its body reserves at the expense of fertility and prolificacy, with negative consequences on the productivity of the farm. Although energy deficit is physiological and largely unavoidable during the first reproductive cycles, it is possible to try to control it focusing on the feeding. It may be useful to increase the concentration of digestible energy in the diet, therefore the amount of starch and lipids (Cerolini et al., 2015).

However, dietary strategies are not enough on their own to solve this deficit, so other operations must be implemented, aimed at:

- increasing the capacity of feed intake, by implementing selection and crossbreed programs
- adopting less intensive production rates, even if with a consequent lower number of kindlings/year
- anticipation of the kits weaning age (21-25 days of age) to reduce milk production (difficulty of application due to negative consequences on kits welfare) (Cheeke et al., 1987).

2.4 Artificial insemination practice

The AI practice in rabbits appeared on European farms in the late 1980s.This reproductive technology has allowed the development of a new cycled production system and a better organization of farms. In France, for example, farmers generally buy heterosperm pools from one of the 20 production centers, carrying out insemination at their farms (Theau-Clément, 2007).
The main reason why this technique is used in farm animals is to accelerate the rate of genetic improvement by increasing the productivity of food-producing animals. This was achieved by using semen of highly selected breeders to fertilize thousands of females (Ombelet and Robays, 2015).

The main advantages brought by AI are:

1) insemination of a greater number of females starting from a single ejaculate,
2) reduction of the number of males needed in the farm,
3) improvement of individual fertility through programmed inseminations and hormonal treatments,
4) synchronization of reproduction and kindlings, evaluation of semen quality,
5) reduced risk of venereal diseases (Gamberini, 2001).

AI is a technique that aims at inducing pregnancy even in rabbit does that refuse natural mating (Theau-Clément, 2007). The percentages of conception following AI can be equivalent or better than those achieved with natural mating, with the added benefit of avoiding contact between animals (Morrell, 1995).

2.4.1 Factors that influence the insemination practice

Although rabbit does can be inseminated immediately after kindling, reproductive performances varies considerably from subject to subject, being conditioned by both external and internal factors (Theau-Clément, 2007).
The success of the AI intervention is influenced by the receptivity of the female, in turn due to environmental and individual factors, in particular in relation to the hormonal structure. It follows that to favor the receptivity of the female it is possible to use hormonal treatments, thus obtaining a second advantage, namely the synchronization of estri and therefore of insemination (Gamberini, 2001).

Individual factors:

1) Parity order

There is a negative correlation between the age of the rabbit doe and the rate of conception (Ajuogu and Nyeche, 2015). Nulliparous females, which are usually very receptive, are characterized by greater fertility but less prolificacy. The primiparous inseminated during their first lactation, on the other hand, are generally less receptive and fertile, but have a larger litter size than the first ones. Finally, multiparous have high levels of fertility and large kits size, but these can be variable from the second birth onwards (Theau-Clément, 2007).

2) Physiological status

The receptivity is maximum immediately after kindling, it decreases in the following days to then increase again in a progressive way after one week from the kindling (Gamberini, 2001).

Rabbits, together with the cow and the mare, are the only animals of zootechnical interest for which farmers must provide for a simultaneous management of lactation and gestation. Lactating females are generally less receptive. The effect of the physiological state varies with the lactation stage (Figure 12). On the one hand, these differences are probably associated with the depressing effect of lactation on ovulatory capacity, despite the pharmacological induction of ovulation due to the physiological needs of the species (mating-induced ovulation) at the time of insemination. On the other hand, they are also associated with the increased percentage of gestation failures, independently from ovulation (Theau-Clément, 2007).

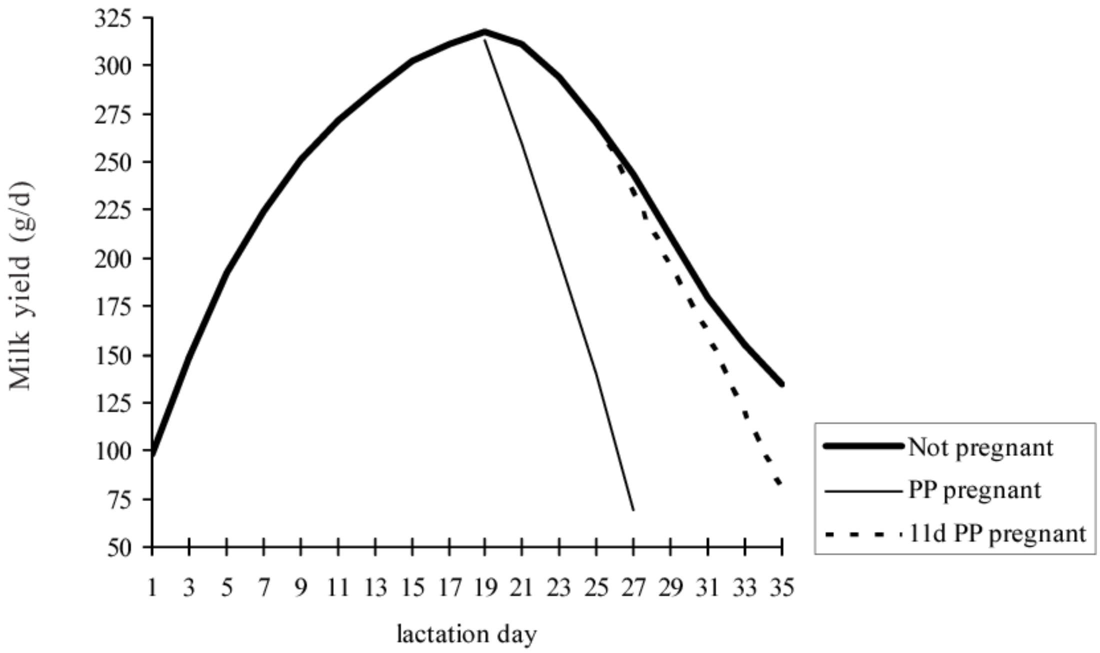


Figure 12. Rabbits does lactation curve according to their physiological status: antagonism between lactation and reproduction (Maertens and Szendro, 2006).

External factors:

1) Proper detection of oestrus

The poor expression of behavioural signs in this species and the nature of physical changes in the reproductive tract make it difficult to determine the exact and precise moment for insemination, since the fertilization procedure may not initiate a sufficient neural stimulus to induce ovulation, compared to the coital activity of the buck (Ajuogu and Nyeche, 2015).

The sexual receptivity of the doe is easy to assess when natural mating is applied. In fact, all rabbit does that accept mating are sexually receptive, while when AI is performed, two methods can be used to recognize receptivity: the test of sexual behaviour or evaluation of vulvar color and turgor. The analysis of sexual behaviour consists in presenting each female before AI to two bucks: these are considered receptive if they take a lordosis position (Menchetti et al., 2015). Regarding the color of the vulva, in nulliparous females, who are generally receptive, fertility rates are quite similar regardless of vulva color. In multiparous, the number of post-AI fertile females is significantly higher when the color of the vulva is red or purple, compared to when the color of the vulva is white/pink. However, the inability to conceive with a pale or white vulva is well known even after natural mating (Ajuogu and Nyeche, 2015).

2) Seasonality

Rabbit is a species characterized by increasing photoperiodism. This means that fertility is at its peak during periods of increased daylight hours (Theau-Clément, 1998).

Light is important for crepuscular or nocturnal species such as wild rabbits: they are able to see well at night; however their color vision is limited. Rabbits are exposed to a periodic light-dark environment that generates a 24-hour (circadian) rhythm. When rabbits live in periods of light or continuous darkness, the daily rhythms are respectively longer or shorter than 24 hours. The activity of European wild rabbits depends on the season and the time of sunrise and sunset (Szendrő et al., 2016). Under wildlife conditions, the breeding season lasts from February to September, but could extend longer (Smelser et al., 1934).

This seasonal rhythm is more pronounced in wild animals, while it is more or less attenuated in farm conditions. The activity of farmed rabbits depends on the hours in which the lights are switched off and on. Seasonal effects are limited when lighting is applied for 16 hours. The change from 8 to 16 light hours, eight days before the insemination, is effective to increase the receptivity and the pregnancy rate (Szendrő et al., 2016).

In general, therefore, the seasons of the year influence both natural mating and artificial insemination. The highest conception rate is reached between April and May and the lowest between August and September. Seasonal fluctuations are also lower for rabbits inseminated after weaning than those inseminated before weaning (Ajuogu and Nyeche, 2015).

2.5 Reproductive rhythms

Intensive rhythm

Intensive reproductive rhythm refers to AI performed immediately after kindling (Cerolini et al., 2015).

For several decades, the production of rabbits in developed countries has been intensified as the number of animals per farming unit, the productivity of the females and the feed conversion ratio have greatly improved. The reason for this improvement could be mainly sought in the development of cycling systems, combined with artificial insemination which improved the efficiency of work on the farm, genetic selection and a better knowledge of the nutritional needs of animals (Theau-Clément et al., 2016).

Dietary strategies are frequently based on choosing a high-energy diet for does subjected to an intensive rhythm, but often this is not enough to guarantee them a good body condition. The application of this rhythm, in fact, means having females who are simultaneously pregnant and lactating for more than half of the reproductive cycle. In the current reproductive rhythms, the wide overlap between lactation and gestation, and the consequent energetic and hormonal antagonism, reduces the fertility rate and the life span of the female, due to the inability of responding to the high nutritional requirements (Castellini, 2007).

It has been shown that reducing the nutritional requirements of females, limiting the overlap between lactation phase and pregnancy, allows a lower mobilization of lipid reserves in primiparous animals (Theau-Clément et al., 2016).

The intensive rhythm also influences serum concentrations, both of estradiol and testosterone, together with the quality of the oocytes, leading to a decrease in reproductive parameters (Garcia-Garcia et al., 2009).

Regarding the aspect of sustainability, this system undoubtedly improves economic profitability (the does's reproductive capacity is maximized), which can be seen with a greater number of reproductive cycles per year. However, this causes a negative effect on the efficiency of the reproductive process and on farming practices respectful of animal welfare, resulting in such a high rate of annual replacement that it is not economically viable (Lavazza et al., 2009).

Semi-intensive rhythm

At the moment, it is the most widely reproductive rhythm used at European level and represents a compromise between the intensive and the extensive system. In fact, females are artificially inseminated every 42 days (=11 days *pp*). This management allows a highly efficient organization in the farm (Theau-Clément et al., 2016).

Extensive rhythm

This system involves the use of post-weaning AI (Cerolini et al., 2015). In an economy that aims at reaching the highest levels of production, the farming realities should be able to propose new models that are sustainable from an economic, social and ecological point of view (Fortun-Lamothe et al., 2010). Resorting to this rhythm certainly means taking into account, in a more respectful way, the reproductive physiology of the female, reducing for them the reproductive pressure (Fortun-Lamothe et al., 2010). With this system the exploitation of does is less intense and thus allows a rate of recovery of 70-80% against 130% of the semi-intensive system (Lavazza et al., 2009).

Theau-Clément and her collaborators (2016) recently compared three breeding systems that differed regarding the reproductive rhythm, the age of females at first AI, age of kits at weaning and slaughter, and the extensive system resulted to be the best in terms of fertility, litter size and number of live born kits.

2.6 Ovulation induction

In rabbit does, the act of coitus triggers a neuro-endocrine reflex and an afferent stimulation that reaches the midbrain and the diencephalon. The stimuli arising from the vaginal region, as well as the olfactory signals and the tactile stimulation of the buck, activate the Gonadotropin-releasing hormone (GnRH) neurons that cause the GnRh release in the median eminence. This peptide then binds to similar receptors in the anterior pituitary, triggering the Luteinizing Hormon (LH) peak which, in turn, activates the mechanisms that cause ovulation (Maranesi et al., 2018). Consequently, during AI practice, since there is no stimulation resulting from mating, ovulation must be induced by artificial stimulation (Quintela et al., 2004).

Ovulation can be induced by two main systems: the first non-pharmacological (biostimulation) and the second pharmacological. Biostimulations are still under study because of the conflicting results obtained, but they are destined to take hold above all in organic farming where the use of drugs is forbidden (Noakes et al., 2009). The pharmacological approach is the one most frequently used because it is more practical and standardized. It is based on hormonal treatments, in particular on intramuscular administration of GnRH analogs. Given at the time of insemination, they determine, at a pituitary level, the release of LH responsible for ovulation (Rebollar et al., 1997).

2.6.1 Intramuscular treatments

1) Pregnant Mare Serum Gonadotropin

Some hormones can be administrated simultaneously to the whole group of rabbit does to obtain the synchronization of the oestri, and therefore of the whole reproductive cycle, with the positive implications on the management of all the phases in the farm. Among these hormones, there is the pregnant mare serum gonadotropin (PMSG), also used to obtain ovulation in rabbits, and therefore the achievement of an optimal physiological situation for fertility purposes (Gamberini, 2001).

Also referred to as equine chorionic gonadotropin (eCG), it is a gonadotropic hormone produced in the chorion of pregnant mares. This molecule is a glycoprotein with a mass estimated between 45 and 64 kDa, having both action as a follicle stimulating hormone and luteinizing hormone. Its main follicle stimulating effect has been used to induce and increase the number of follicles at the time of ovulation (superovulation) first in the cow, then in laboratory animals including the rabbit (Theau-Clément, 2007).

This treatment leads to a significant increase in sexual receptivity, prolificacy (around 5-10%)

and total weight of the litter (Cerolini et al., 2015), as well as in ovulation and fertilization rate (Theau-Clément, 2007). The type of administration, intramuscular or subcutaneous, does not affect its effectiveness (Cerolini et al., 2015).

The rate of ovulation can be improved by following the eCG treatment with the administration of GnRH at the time of AI (Noakes et al., 2009). However, PMSG may have an important immunogenic role, being an exogenous high molecular weight protein. For this reason, its effectiveness may decrease if used for a long time in the rabbit: the answer is however individual (Theau-Clément, 2007).

In general, it can be stated that its effectiveness is conditioned by various factors, such as: dose, physiological status of the female and rate of administration; in particular it is less effective in dry or non-receptive does. Furthermore, it is not immune to some undesirable side effects that may eventually lead to an increase in perinatal mortality and in certain diseases of the female reproductive system (Gamberini, 2001).

2) Prostaglandins (PGF2α)

The luteolytic effect of prostaglandins (PGF2α) is exploited to induce and synchronize kindlings or to induce the regression of corpus luteum in case of pseudopregnancy (Theau-Clément, 2007). An indirect effect of the administration of PGF2α is the increase in sexual receptivity and fertility, starting as early as 7 days after kindling (Cerolini et al., 2015).

Considering their luteolytic action, it can be thought that the efficacy found is linked to the presence in the farm of females with luteus bodies (therefore high concentration of progesterone), whose regression induces the action of estrogens. PGF2α has a lower cost than PMSG and has no contraindications in terms of immune response and has a shorter life-span. The only disadvantage that limits its use for the synchronization of the oestri is that its effect depends on the ovarian, and therefore hormonal, situation at the time of treatment, which in turn modulates the physiological response. As a result, the results are difficult to standardize (Cerolini et al., 2015).

For kindling synchronization, prostaglandins are administered at the end of pregnancy (around the 28th-29th day); it follows, within one or three days, that kindling occurs. In addition to this effect, they also have the advantage of improving receptivity for the subsequent reproductive phase (Gamberini, 2001). On the other hand, interruption of pseudo-pregnancy is obtained by treating the rabbits, which are negative at palpation (12 days post AI), with 200μg of PGF2α (alone or with 25 IU of PMSG) (Cerolini et al., 2015).

The effect of prostaglandins would therefore be indirect, while PMSG would act directly at the ovarian level: by stimulating the growth of follicles, the secretion of estrogens and, consequently, the sexual receptivity of the rabbit is increased. The complementarity of the two hormones could justify their combined use to improve the productivity of the farm (Cerolini et al., 2015).

3) GnRH analogues

Similar-GnRH hormones can be ascribed in two groups: natural (Gonadorelin) and synthetic analogues. In recent years, the technology applied in the biological field has allowed us to produce about 2000 GnRH syntethic analogues, which are variable due to receptor affinity, *in vivo* absorption, resistance to degradation and mode of elimination. A fundamental characteristic of them is the replacement of L-isomers with D-isomers. The most common GnRH synthetic agonists on the market are: Buserelin, Leuprorelin, Goserelin and Triptorelin. In general, they are sensitive to peptidases and therefore to gastrointestinal degradation, making oral administration inadvisable (Conn and Crowley, 1991). Other authors have found a lower intestinal degradability of the analogues compared to natural GnRH (Berger et al., 1991).

A single injection of GnRH causes an initial stimulation of the pituitary cells, with consequent secretion of FSH and LH and with the consequent gonadal response (Figure 13). However, the continuous or repeated administration or the administration of supraphysiological doses, determines the inhibition of the pituitary-gonad axis with consequent decrease in steroidogenesis and gametogenesis (Conn and Crowley, 1991).

Studies carried out by Yoshimura and his collaborators in 1992 have shown that the intramuscular administration of GnRH or its synthetic analogues induces ovulation in a similar way to what would occur with natural mating. It must be emphasized that these hormones directly influence the ovarian functions, in particular the maturation of the oocytes, both in *vivo* and in *vitro*.

In addition to inducing ovulation, the use of GnRH analogues also allows to improve some reproductive parameters. Zapletal and Pavlik (2008) showed that the amount of hormone administered has a positive influence on the reproductive performance of the doe, in particular on the number of newborns per litter and on the conception rate: as the dose increases, the number of kits/doe increases.

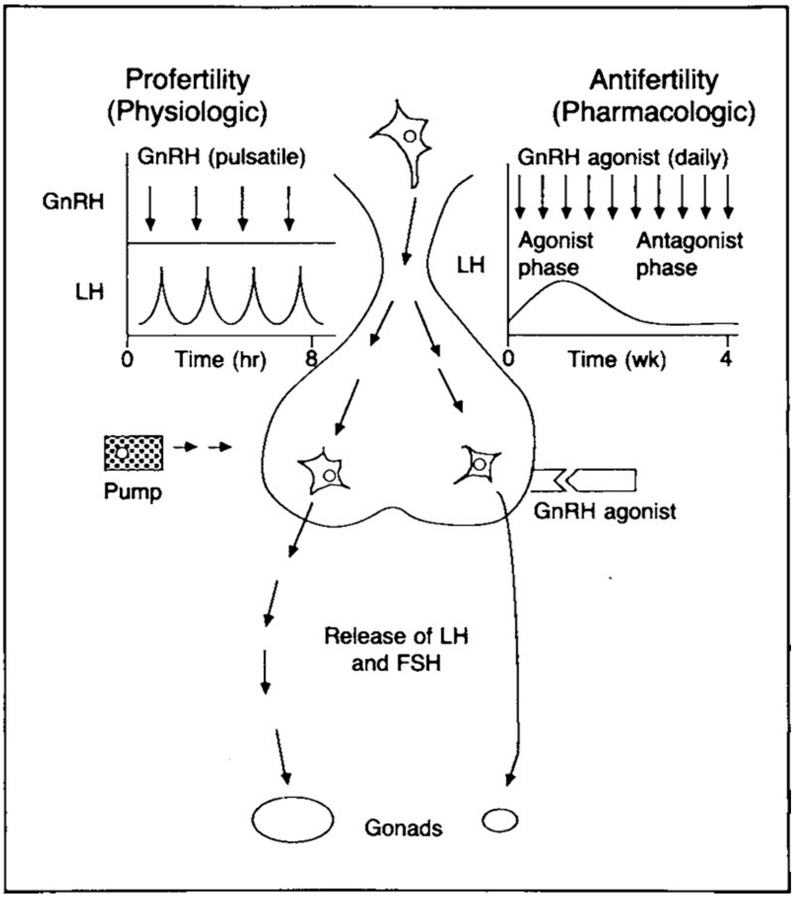


Figure 13. Modes of action of GnRH and its long-acting agonist analogues.

2.6.2 Intravaginal treatments

The GnRH intramuscular administration method, particularly when repeatedly used in rabbit does, is generally followed by a decrease in fertility due to the appearance of plasmatic anti-GnRH antibodies (Theau-Clément et al., 2008). Under commercial farm conditions, rabbit does are routinely administered intramuscular treatments, but this process is invasive and best practice guidelines are not well developed (Castellini et al., 2007). The European Food Safety Authority (EFSA, 2005) currently recommends that hormone treatments have to be used in a limited manner, as infrequently as possible, and that they have to be replaced, if possible, with alternative methods having no animal welfare consequences. The available evidence suggests a need to improve the welfare and fertility parameters of rabbit does (Castellini et al., 2007) through the development of new reproductive strategies to induce doe ovulation, avoiding the traumatic event of intramuscular administration.

Several authors demonstrated ovulation induction by including different GnRH analogues in the seminal dose, and administering them through vaginal absorption (Quintela et al., 2004, 2008; Dal Bosco et al., 2014). Zhang and Qin (2012) confirmed that inclusion of the GnRH analogue leuprorelin in the seminal dose induced doe ovulation, and led to the same reproductive performance obtained with the intramuscular method. Moreover, GnRH intravaginal administration could be beneficial for farmers, avoiding potential mistakes derived from incorrect hormone administration and reducing the time spent on each AI (Quintela et al., 2004).

There are some factors that can influence the GnRH absorption e.g. the state of the vaginal mucosa (in particular secretions related to the receptivity of the female), the composition and ability of the sperm to incorporate foreign molecules (Quintela et al., 2008). The cell layers of the vagina have enzymatic activity and, among them, proteases are likely to be the important barrier for the absorption of peptides and proteins. Perhaps it is one of the reasons why it is necessary to increase the intramuscular dose of Buserelin by about 10 times, when it is included in the intravaginal seminal doses (Ondruska et al., 2008).

3. AIM OF THE PHD PROJECT

Under intensive rabbit farming, the most widespread reproductive rhythm practiced is based on artificial insemination performed at around 11 days *postpartum* simultaneously with intramuscular administration of GnRH to induce ovulation, which involves double containment of the animal. This commercial breeding system is well adapted to cycled production, but it could not take into account the reproductive physiology of the doe, so it results in high production rates, but requires high yearly replacement of animals that cannot sustain the energy demand. Moreover, current housing systems do not allow animals to express the typical behavioural pattern of the species reducing welfare, with presence of abnormal behaviours. The combination of this different factors (invasive techniques, intensive rhythm and reduced housing space) can contribute to a reduction in rabbit does reproductive carrier, being not able to cope the cronic stress along reproductive cycles.

Housing animals in cages more respectful of their behavioural repertoire, together with the extensification of the reproductive rhythm and animal-friendly practices could allow better animal welfare, with a positive impact also on performances and then on farmer income.

Having the above mentioned as guidelines, the present PhD Thesis was conformed with the goal of proposing alternative solutions (housing, practices and rhythm reproductive) for rabbit doe farming, that can be adopted under intensive conditions in order to have an improvement in welfare and consequently an extension of the reproductive career of rabbit does.

EXPERIMENTAL SECTION

4. MATERIALS AND METHODS

4.1 Experiment 1: Effects of an intravaginal GnRH analogue administration on rabbit reproductive parameters and welfare

Animals, experimental groups and feeding

This study was carried out at Cascina Campora commercial rabbit farm in the period between October 2017 and May 2018. The company was located in the heart of Monferrato in 1988, in Buttigliera d'Asti (Piedmont, Italy). The breeding is a closed cycle, so it has both reproduction and fattening periods. The control of the main diseases, gastrointestinal and respiratory (coccidiosis, pasteurellosis, etc.) typical of the rabbit, takes place through regular vaccination and constant prevention. The space for rabbits is located inside a barn: the farming require daily monitoring of the environmental temperature (15-28°C) and relative humidity (60–75%). Ventilation is controlled by automated systems (0.3 m / sec). The lighting regime adopted is based on the use of 16 hours of light and 8 hours of dark, to obtain the best results in terms of receptivity and fertility (Cerioli et al., 2008). The animals received a commercial diet containing 16.9% crude protein, 14.2% crude fiber, and 3.5% fat. Food and water were provided ad libitum throughout the experimental period. All animal experiments were managed in accordance with the Turin University Bioethics Committee recommendations (Prot. N° 256053 of 4/07/2017).

The experimental animals included 20 9-month-old rabbit does, each having two previous deliveries (Grigio del Monferrato, autochthonous Italian breed). These animals were single-cage housed and divided into two experimental groups. The control group (C; n=10) received 0.2 mL of intramuscular lecirelin acetate (Dalmarelin, Fatro®, Italy), and was then inseminated with normal extended semen. The intra-vaginal group (IV; n=10) was inseminated with an extended seminal dose including 0.3 mL lecirelin acetate (Dal Bosco et al., 2014). All does underwent AI for six consecutive cycles, using a seminal dose containing 10 ± 1 million spermatozoa in 0.5 mL of diluent (Castellini and Lattaioli, 1999). No estrus synchronization was performed.

Reproductive performances

Rabbit does were managed according to a cycled production system with a 42-day interval and a dark/light program according to Mousa-Balabel et al. (2011). We recorded the following reproductive traits at A.I.: sexual receptivity (vulva color and turgescency; a doe was deemed receptive when its vulva was red or purple and turgid), fertility rate (kindling/inseminations × 100), and number of live-born kits (Castellini et al., 2005).

Semen collection and evaluation

For each reproductive cycle, one ejaculate per male (n=3) was collected early in the morning on a single day using an artificial vagina (Boiti, 2005). Only ejaculates exhibiting a white color were used, and if the gel was present, it was removed. Ejaculates with over 70% motile sperm were pooled (54 heterospermic pooled semen) and used for AI (Lavara et al., 2005). Each ejaculate pool was divided into two samples, which were diluted 1:5 using Galap (IMV Technologies, L'Aigle, France) (Brun et al., 2002), with addition of Dalmarelin (IV group) or without any additional hormone (C group). The solvent of Dalmarelin (physiological solution 0.9% NaCl) did not affect sperm quality (Blaszczyk et al., 2013). Samples were assessed for motility characteristics, sperm viability, and acrosome status.

Assessment of semen motility characteristics, sperm livability, and acrosome status

Sperm motility and motility characteristics at 37°C were evaluated using a computer-assisted sperm analyzer (CASA; Hamilton Thorne, Inc., Beverly, MA, USA) with a 10× objective. A 10-μL specimen of diluted semen was put on a pre-warmed Mackler slide and evaluated. Motility values were recorded as the percentages of progressive motility (P. MOTIL, percentage of sperm exhibiting an actual space gain motility) and total motile sperm cells (TOTAL MOTIL). Additionally, based on the frequency with which the sperm track crossed the cell path in either direction and in changeable tracks (Lukac, 2009; 2011), we calculated the average path velocity (VAP, μm/s), straight linear velocity (VSL, μm/s), curvilinear velocity (VCL, μm/s), amplitude of lateral head displacement (ALH, μm/s), linearity index (LIN, average value of the VSL/VCL ratio, %), straightness index (STR, average value of the VSL/VAP ratio, %), and beat-cross frequency (BCF, Hz).

Sperm viability and acrosome status (RSA) were evaluated using the Trypan blue/Giemsa dual staining technique, as previously described (Kovacs, 1992; Boccia et al., 2007). Trypan blue was used first to differentiate live from dead spermatozoa. Then the dried smears were fixed in 37% formaldehyde and stained with Giemsa for acrosome evaluation using an Advanced Automated Research Microscope System (Nikon Eclipse E200, phase contrast at 40 and 100 magnifications). We counted at least 200 sperm cells for each group. Acrosome-intact live (AIL) spermatozoa were differentiated based on staining characteristics. Only sperm displaying both head and tail were recorded as viable, while those with only either the head or the tail were considered unviable.

Statistical analysis

Statistical analyses were performed using SPSS statistical package version 16 (SPSS, Chicago, Illinois, USA) with one-way analysis of variance (ANOVA): descriptive statistics were used to prove the significant differences in seminal parameters including sperm motility, livability and acrosomal status (expressed as mean ± SE) and reproductive parameters including receptivity, fertility and live-born kits (expressed as mean ± SD) between the 2 experimental groups (C and IV group) during the different insemination cycles (each parameter was considered as a dependent variable, while group and cycle as fixed factors). Multiple comparisons of the means were done with Duncan test and P value was set at <0.05.

Multiple regression analysis was performed to develop a model for evaluating the correlation coefficients between sperm parameters and doe reproductive characteristics; Pearson's coefficients were calculated to assess the correlation between sperm and reproductive parameters; only fertility showed significant differences. P value was set at <0.05 to indicate statistical significance.

4.2 Experiment 2: A multifactorial evaluation of different reproductive rhythms and housing systems for improving welfare in rabbit does

Animals and experimental design

The study was performed at Cascina Campora commercial farm in Buttigliera d'Asti (AT), Italy, from October 2018 to December 2019. All animals were handled in accordance with the Turin University Bioethics Committee recommendations (Prot. N 256053 of 4/07/2017).
A total of 110 rabbit does (77 days old) of the Grimaud strain (hybrid maternal line, homogenous for genetics and age) were randomly divided into one of two housing systems, as follows:

- 60 nulliparous does were housed in 15 Combi (C) cages (WRSA, Combi 6®; Meneghin S.r.l, Povegliano, TV, Italy). The main characteristics of these cages were: the dimensions (97.5 cm long x 113 cm high), the plastic platform, the absence of a top cover, and the mobile walls, which can allow the group housing of 4 does with their kits. This C cage was considered an autonomous, independent production unit (Figure 14). In this trial, we tested the C cages without removing mobile walls to exclude the effects of social interactions.
- 15 nulliparous does were housed in Standard cages (S). These cages were 60 cm long x 35 cm high, with an external nest box. Each S cage was considered an autonomous, independent production unit.
- the remaining 35 does were maintained for replacements.

Nulliparous does were weighed weekly, from the day of arrival to the 19th week. Weight gain was controlled up to the first artificial insemination (AI, at a live weight of 4061 + 334.15 g, at 19 weeks of age) to have homogeneous animals. Both the C and S groups underwent 6 consecutive AI cycles.

At the second AI, all C and S does were allocated to one of three experimental subgroups, with different reproductive rhythms, as follows:

- Intensive (I): AI at 11 days postpartum;

- Alternating (A): AI at 11 days postpartum, alternated with AI after weaning (30 days postpartum);
- Extensive (E): AI performed after weaning (30 days postpartum).

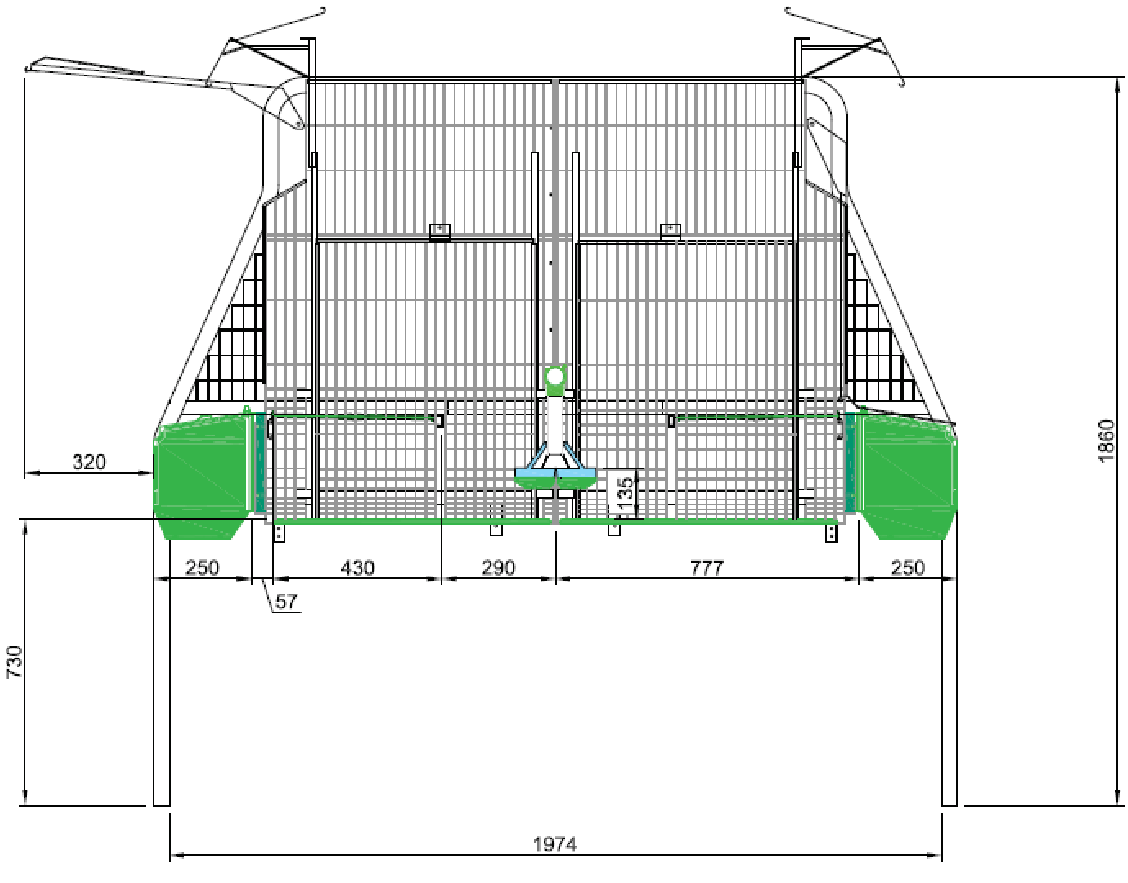
The corresponding balanced (2x3) experimental plan, was composed of:

- CI group = 20 C does submitted to an I rhythm
- CA group = 20 C does submitted to an A rhythm
- CE group = 20 C does submitted to an E rhythm
- SI = 5 S does submitted to an I rhythm
- SA = 5 S does submitted to an A rhythm
- SE = 5 S does submitted to an E rhythm.

for a total of 6 consecutive reproductive cycles.

At 12 days after AI, all animals were manually palpated, and animals that were not pregnant were re-inseminated according to the corresponding initial rhythm.

Rabbits were kept in controlled conditions; the environment was maintained at 15-18 °C and 60-75% relative humidity. The barn was artificially ventilated (airflow, 0.3 m/s) and ambient light followed a natural photoperiod. The rabbit does received a commercial diet containing 16.9% crude protein, 14.2% crude fibre, and 3.5% ether extract.



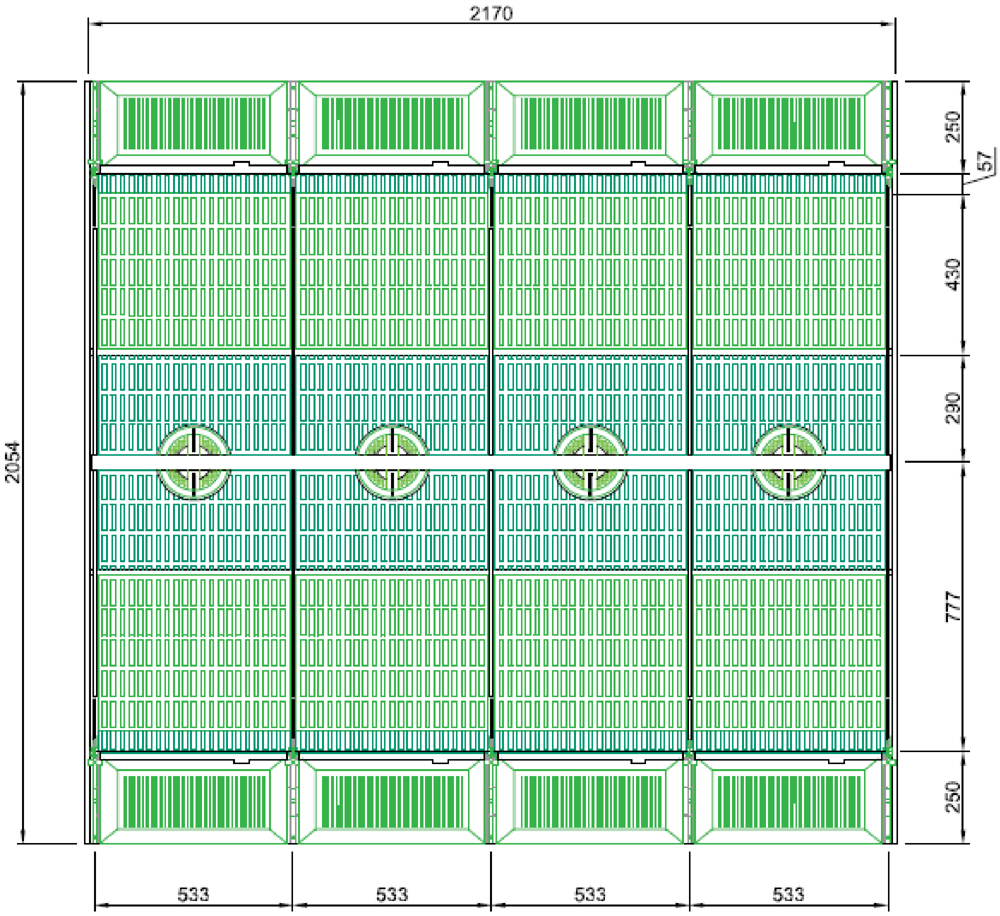


Figure 14. Scheme of Combi cage

Reproductive and productive performances

AI was performed in the morning by inseminating with 0.5 mL of diluted fresh semen, containing about 10 million spermatozoa (Castellini and Lattaioli, 1999). Oestrus synchronization was not performed. Ovulation was induced before AI by inoculating each doe with 0.2 mL lecirelin intramuscularly (Dalmarelin, Fatro®, Italy). At 12 days after AI, manual palpation was performed to assess pregnancy. The following reproductive/productive indices were recorded: doe weight at AI, sexual receptivity (based on vulva colour and turgescence: a red or purple vulva that was turgid indicated receptivity), and fertility rate (calculated as the number of kindlings/inseminations □100). At kindling, we recorded the number of live born kits/litter, the number of dead kits/litter, the number of weaned kits, the weight of the weaned litter, the average kit weight at weaning (weight of weaned litter/total number of weaned kits), and the pre-weaning mortality (number of dead kits/number of weaned kits) (Castellini et al., 2003).

The indices of efficiency were the overall productivity (number and weight of rabbits sold/year/doe) and production losses (difference between actual and theoretical production, assuming fertility rate = 100, mortality of the young rabbits = 0, and kindling interval = 60) (Castellini et al., 2005).

Controlled nursing was performed until 16 days after birth. Does were permitted access to the nest only once per day for 15 min (Castellini et al., 2003).

Body Condition Score

The body condition score (BCS) was evaluated at each AI in all the females. The scoring was based on palpating the loin and rump regions. The loin was felt for vertical bone protrusions (spinous process) and the fullness of the muscle over and around the vertebrae; the rump was felt for bone protrusions and the fullness of the muscle. The loin was subjectively evaluated according to poor, intermediate or wide level, whereas the rump for poor or wide level. The BCS was “0” when the loin was rated poor; “1” when the loin was rated intermediate and the rump was rated poor; or “2” when the loin was rated intermediate or wide and the rump was rated wide (Bonanno et al., 2008).

Behaviour recordings

Among 39 rabbit does (10 does/rhythm in the C system and 3 does/rhythm in the S system), behavioural patterns were recorded with direct observations in the time window between 8.00 a.m. and 16.00 p.m. by two experienced operators that had been previously trained together. Simultaneous training was conducted to guarantee accurate inter-observer agreement (Cohen, 1960). We applied the Focal Animal Scan Sampling Method (Martin and Bateson, 1986). Behaviours were evaluated during all reproductive cycles, at three important times: 2 days after AI, 2 days after birth, and 2 days after the lactation peak, for a total of 6 days/cycle/group. Data were reported on a designated form. Before each observation, animals were allowed to adapt to the presence of operators for 5 min. To establish the end of a performed behaviour, the operator waited for 10 s to determine whether the same behaviour was repeated; after the 10 s, any behaviour was recorded as a new behaviour (Bornett et al., 2000).

To develop the ethogram (Table 1), the following behaviours were recorded: static activities (crouching, staying, sitting up, or lying down, all with the ears down), static in alert (crouching, staying, sitting up, or lying down, all with the ears erect), standing alert, moving (running, exploring, jumping), self-grooming, feeding, drinking and stereotypical behaviours (sniffing and biting bars) (Figure 15).

We also assessed the inter-observer reliability index (Cohen’s kappa): all behavioural results were found to be extremely accurate, confirmed by the Cohen’s kappa reliability index of 0.97, where 0 indicates no accuracy and 1.00 indicates the maximum accuracy. In all experimental groups, does were observed for a total of 4212 min (117 min/day x 6 days of observations x 6 reproductive cycles). For each animal, all specific behaviours were expressed as a percentage, calculated as: the number of times it occurred/the total number of observations x 100.

Table 1. Categories of behavioural patterns with description.

	Behaviour patterns	*Behaviour description*
Static	Crouching with ears down	Resting with chest on the floor. Hind and fore limbs crouched under body
	Staying with ears down	Stand still on the 4 straight limbs
	Sitting-up with ears down	Sat in upright position on hind limbs and fore limbs straight, but not bust touch the floor
	Lying down with ears down	Resting with chest on the floor. Fore limbs stretched in front of the body
Static in alert	Crouching with erect ears	Resting with chest on the floor. Hind and fore limbs crouched under body. Ears are erect
	Staying with erect ears	Stand still on the 4 straight limbs. Ears are erect
	Sitting-up with erect ears	Sat in upright position on hind limbs and fore limbs straight, but not bust touch the floor. Ears are erect
	Lying down with erect ears	Resting with chest on the floor. Fore limbs stretched in front of the body. Ears are erect
Standing alert	Standing up on the hind legs with erect ears	Sat in upright position. Ears are erect
	Standing up on hind legs with ears down	Sat in upright position on hind limbs and fore limbs straight.
Moving	Running	Any movement in any direction where all four limbs are involved
	Exploring	Any movement that involves sniffing activities
	Jumping	Voluntary movements of jumping (almost 3)
Self-grooming	Comfort	Licking, scratching, or nibbling of the body
Eating	Feeding	Head above the feeder. Eating or chewing pellets
	Drinking	Head in close proximity to water nipple. Nosing or drinking from water nipple
Stereotypies	Biting bars	Licking or gnawing cage bars and scratching cage floor insistently
	Sniffing bars	Smelling bars and cage floor insistently

Figure 15. Comby cage equipped with an elevated platform

Maternal attitude and tonic immobility test

The maternal attitude at kindling was evaluated by assessing the quality of the nest. This qualitative analysis (Blumetto et al., 2010) included the following items: the level of mixing between nest material (straw) and the doe's hair (1: no evidence of mixing; 2: an important level of mixing; or 3: almost all the material was mixed); the amount of visible hair in the nest (1: no hair visible; 2: hair visible in more than 50% of the nest ; 3: nesting material not visible in more than 50% of the nest; 4: only hair visible); and the amount of original nesting material preserved in the nest (1: less than 30%; 2: 30% to 60%; 3: over 60%).

The tonic immobility (TI) (Figure 16) test was performed using the same 39 rabbit does for which behaviour was observed (10/rhythm in the C system and 3/rhythm in the S system). The TI test was repeated with the same animals at three times during each reproductive cycle: at insemination, at kindling, and at the peak of lactation. The operator removed the rabbit from the cage and induced immobility by turning the animal on its back while lying on the operator's arms. The immobile rabbit was laid down on a plastic support (Ferrante et al., 1992). A maximum of three attempts were carried out to induce immobility. Animals were not allowed to remain in the immobility condition for more than 10 min. The number of attempts necessary to induce immobility and the total duration of the condition were recorded for each doe

Figure 16. Tonic immobility test

Corticosterone samples collection and hormonal evaluation

We assessed animal welfare by assessing a key physiological indicator, the saliva corticosterone (CORT) level (Bennet and Hayssen, 2010). Before starting the experiment, hormone levels were assessed on two days to establish baseline levels. Saliva was collected with saliva collectors® (Sarstedt SRL – Verona, Italy) (Figure 17).

For 6 consecutive cycles, samples were collected in the same does that underwent behavioural and TI tests, at three different times: 1 day after AI, 1 day after kindling, and 1 day after the lactation peak (16 days after birth). Collected samples were immediately frozen and stored at −80 °C until analysis. Subsequently, stored samples were thawed and prepared for hormonal assays. Thawed samples were centrifuged for 2 min at 1000 xg; next, saliva was recovered and diluted 1:4 with Assay Buffer (Arbor Assays, Ann Arbor, US). The CORT present in saliva samples was determined with a multispecies commercial enzyme immunoassay kit (K014; Arbor Assays, Ann Arbor, MI), which was validated for saliva and other biological substrates. Inter- and intra-assay coefficients of variation were <10%. According to the manufacturer, the kit displayed the following cross reactivities: 100% with corticosterone, 18.9% with 1-dehydrocorticosterone, 12.3% with desoxycorticosterone and 0.38 with cortisol. Serial dilutions (1:4, 1:8, 1:16, and 1:32) of saliva samples were assayed to compare to the standard curve ($P<0.05$ for all assays). In addition, CORT was added to saliva samples (n=6) to determine recovery; the mean recovery rate was 96.8%. The results are expressed as the amount of CORT in saliva (ng/mL).

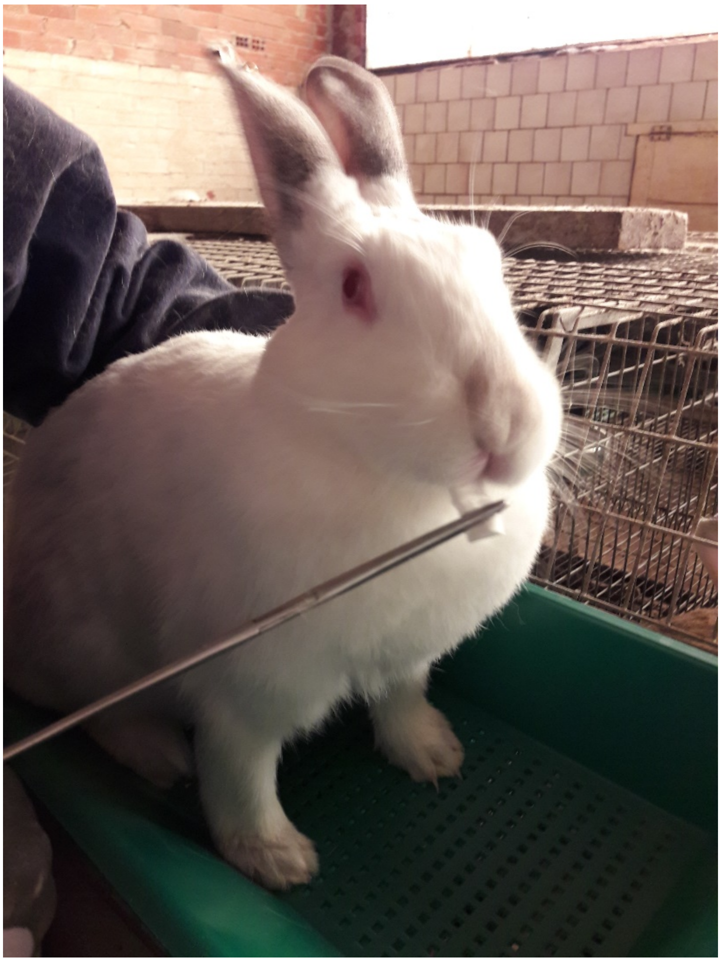


Figure 17. Salivary corticosterone sampling

Statistical analysis

Statistical analyses were performed with the SPSS 16.0 software package. We used the two-way analysis of variance (ANOVA) to evaluate the effects of the housing system, the reproductive rhythms, and their interactions. We found no differences among the three physiological phases and cycles. Therefore, all data were pooled to obtain mean values. Data are presented as the mean ± standard deviation (SD). Multiple comparisons of the means were carried out by calculating the least significant difference with the Duncan test. Statistical significance was set to $P < 0.05$.

4.3 Experiment 3: Effect of different management protocols for grouping does on aggression and dominance hierarchies

Animals and housing

The experiment was carried out in a commercial rabbit farm in Geltwil (Switzerland), using a total of 57 does of the Hycole hybrid maternal line that were not nulliparous, from August 2018 until March 2019. Does were housed in groups of eight animals each, for five consecutive trials (= five consecutive reproductive cycles). They were reared according to a Swiss animal-friendly label programme, which requires group housing of females and a separated nest for each doe (http://www.blw.admin.ch/themen/00006/01715/01718/index.html?lang=de). Each pen was equipped with straw material and furnished with elevated platforms, hiding places, eight compartments with nest boxes, drinkers and automatic feeders (Figure 18). Feed (UFA 925, UFA AG, Herzogenbuchsee, Switzerland), water and hay were provided ad libitum.

Figure 18. Pen design: separate compartment with nest (1), upper wire mesh (2) to isolate compartments, removable nests (3), individual feeder (4) and elevated platform (5).

Experimental timing and management protocols

For each of five trials, all animals were artificially inseminated (AI) on day 10 *postpartum* (*pp*) and were housed individually from one day before parturition until day 11 *pp*. From this point, does were

divided into three different management protocols (MPs) (Figure 19), as follows: group housing from day 12 *pp* (MP12), group housing from day 18 *pp* (MP18), group housing from day 22 *pp* (MP22).
To avoid the effect of parity order, in trial 1 all does were assigned to each MP semi-randomly in a standardized way to ensure a similar distribution of parities. In consecutive trials, doe groups were assigned to another MP. Does not pregnant as detected by manual palpation were replaced with other animals. At least 2 does were replaced in each MP after each trial to achieve the group size of 8. No group remained stable between trials.

Behavioural observations

Following the methods of Andrist et al. (2012), video sequences were recorded and evaluated (Figure 19) for each management protocol, as follows: MP12 at days 12, 18 and 22 *pp*, MP18 at days 18, 24 and 28 *pp*, MP22 at days 22 and 28 *pp*. All groups were observed during the first 24 hours and after 6 and 10 days after regrouping, except for MP22 group in which day 10 was not present because it was after weaning of the kits. All does were individually marked with livestock colour on their backs and had numbered ear tags.

In accordance with Selzer et al. (2001), active behaviour is more common during dark hours than during light hours, because rabbits are crepuscular animals. Therefore, two time-windows of 4 h each between 20:00 and 00:00 (dusk and night) and between 04:00 and 08:00 (night and dawn), respectively, were analysed for each time point through video recordings with infra-red sensitive cameras. Based on an ethogram by Graf et al. (2011), aggressive interactions were classified as biting (gripping with the teeth), boxing (hitting with the front paws), chasing (aggressive following of another individual for at least three jumps), ripping (two does kicking each other with the hind legs), carousel-fights (rapid chasing around and around in one spot with the rear end of the opponent gripped between their teeth), threatening (quick head movement towards another doe) and attacking (abruptly running towards a group mate). Threatening and attacking were combined as mild aggressions because no body contact and hence no injuries resulted. Likewise, biting and ripping were combined into a single response (severe aggressions) as were chasing and carousel (without biting) (chasing behaviour).

For each agonistic event, the following parameters were recorded: type of aggressive interaction, frequency of occurrence, the animal directing the behaviour (dominant subject), the recipient of the behaviour (submissive animal), and location (classified as own nest, foreign nest, platform, down) (Williamson et al., 2016). Frequencies of all interactions and durations of chasing, ripping and carousel-fights were recorded. Aggressive interactions were considered to have ended when each individual separated and engaged in different behaviours such as self-grooming, feeding etc.

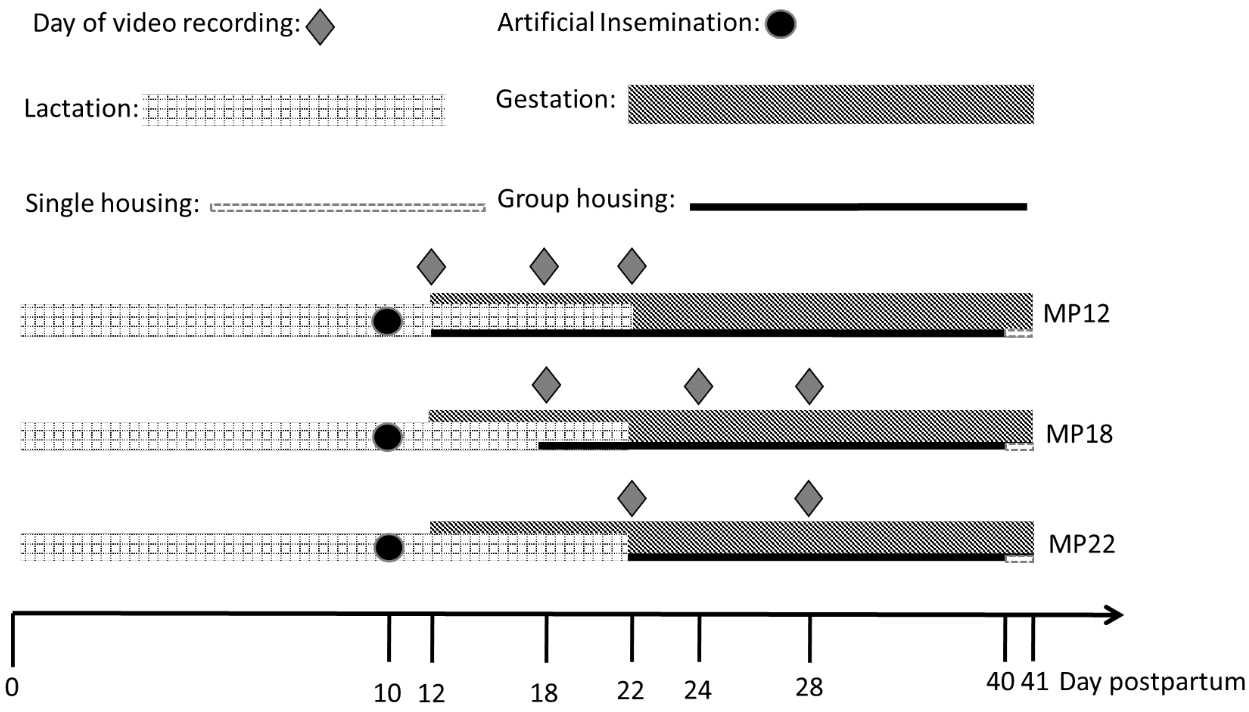


Figure 19. Experimental timing: video recordings, artificial insemination, lactation, gestation, single and group housing of different MPs. MP12: grouping on day 12 *postpartum* (*pp*), MP18: grouping on day 18 *pp*, and MP22: grouping on day 22 *pp*.

Dominance hierarchy analysis

The analysis of dominance within social animal structure has been a research focus since the beginning of the last century (Schjelderup-Ebbe, 1922) and different methods have been used to determine individual ranks from interactions. Among them, the Elo-rating method (Elo, 1978) tracks rank measures as a consequence of wins and losses in encounters with other individuals: numerically greater ratings indicate more successful competitors. Since Neumann et al. (2011) have published an R function, use of Elo-ratings together with a stability index to model dominance hierarchies has become one of the most useful methods in this field (Mc Donald and Shizuka, 2013).

For assessing the dominance hierarchy of each treatment along the five trials, the index of stability (S) was calculated which ranges between 0 and 1, where 1 refers to stable hierarchies, whereas values closer to 0 indicate more unstable hierarchies (McDonald and Shizuka, 2013).

Statistical analysis

Statistical analysis was conducted using the software package SAS 9.4. Generalized linear models on count data (Poisson distribution) with management protocol, trial, and day as fixed categorical effects were computed using Proc Glimmix [SAS/STAT] Version 13.1 software. Residuals were checked for normality. No videos existed for day 10 after regrouping in MP22 because offspring were weaned before this day which made comparisons with the other protocols not possible. Therefore, two sets of analyses were performed: 1) Comparing days 0, 6, and 10 after regrouping for MP12 and 18 and 2) comparing days 0 and 6 after regrouping for all MPs. P-values were adjusted for multiple comparisons by Tukey-Kramer. The relationship between the number of aggressive interactions and time-of-day was analysed with the regression model including the linear and the square term of time-of-day (Proc Reg). Only time points between 20:00 and 24:00 were included because only 3 aggressive events happened during the second time slot from 4:00 to 8:00. However, the other analyses included all data.

Dominance hierarchy and its stability were calculated with R (version 3.6.0), using the package EloRating (version 0.46.8, https://cran.r-project.org/web/packages/EloRating/index.html accessed 9-9-2019). Results were assessed as significant when $P < 0.05$.

5. RESULTS

5.1 Experiment 1: Effects of an intravaginal GnRH analogue administration on rabbit reproductive parameters and welfare

Sperm morphology and motility characteristics

The C group semen showed improved motility parameters compared to the IV group (P≤ 0.01) (Table 2), including a greater % progressive motility (43.31 vs. 38.34), VAP (106.51 vs. 99.55), VSL (86.71 vs. 76.81), BCF (38.75 vs. 36.11), STR (79.76 vs. 75.65), and LIN (52.99 vs. 46.30). On the other hand, the IV group semen showed an increased VCL (171.37 vs. 167.96) and ALH (6.63 vs. 5.95) compared to the C group semen (P≤ 0.01).

Reproductive parameters

Comparing the reproductive performances of does revealed effects of group and cycle, and their interaction (P <0.001) (Table 3). The IV group showed a significant effect regarding sexual receptivity compared to the C group (P <0.001) along cycles. Within cycles, sexual receptivity was greater in cycles 3, 5, and 6 (100, 100 and 95, respectively) compared to in cycles 1, 2, and 4 (0, 75 and 50, respectively) (P < 0.001).

We found a significant group effect (P <0.001) with regards to fertility rate during cycles 2, 4 and 5 between the does in the C group (60, 60 and 40%) and in group IV (100, 100 and 80 %, respectively). While in cycle 3, the fertility rate was significantly (P <0.001) increased with the C group compared to the IV group (60% vs. 50%). A significant fertility rate was recorded within cycle 1 (60-60%) and cycle 6 (80-80%) with the C and IV groups respectively. Regarding the number of live-born kits (results originated from both groups data), only cycle showed a significant effect (P<0.01), with differences recorded within cycles 2, 4 and 6 (6.45, 8.15 vs. 6.15, respectively) (Figure 20).

Correlation

We identified several significant correlations between sperm motility, morphological parameters, and doe reproductive aspects (Table 4) (data was originated from both groups, C and IV). The % of live sperm showed a positive correlation (0.40, P<0.01) with the % of sperm with intact acrosome. Moreover, progressive motility was positively correlated (P <0.001) with several motility characteristics, including VAP (0.77), VSL (0.87), ALH (0.76), BCF (0.72), STR (0.79), and LIN (0.84). On the other hand, progressive motility was negatively correlated with fertility rate (–0.19, P <0.001).

Regression model

The sperm parameters VAP, VSL, VCL, ALH, BCF, STR, LIN, total motility, progressive motility, and % of live sperm with intact acrosome explained only 4% of the variation in fertility rate (Table 5) (data was originated from both groups, C and IV). Regression analysis revealed that fertility was negatively impacted ($P \leq 0.05$) by VAP (−0.56) and progressive motility (−0.24), and was positively impacted by VSL (0.36).

Table 2. Sperm characteristics in two different experimental groups of rabbit semen.

	Group	
Morphological characteristics of sperms	**Control (C)**	**Intravaginal (IV)**
Live sperm (%)	74.55± 2.31	72.00±1.95
Sperm with intact acrosome (%)	78.65± 1.92	79.00± 1.81
Computer-assisted semen analysis for motility (CASA)		
VAP (μm/s)	106.51± 0.90	99.55± 0.85*
VSL (μm/s)	86.71± 1.23	76.81± 1.22*
VCL (μm/s)	167.96± 0.79	171.37± 1.08*
ALH (μm/s)	5.95± 0.09	6.63± 0.10*
BCF (Hz)	38.75± 0.39	36.11± 0.44*
STR (%)	79.76± 0.62	75.65 ±0.75*
LIN (%)	52.99± 0.85	46.30 ±0.86*
Total motility (%)	60.83± 1.24	60.01± 1.32
Progressive motility (%)	43.31± 1.24	38.34± 1.29*

Based on means ± SE of 54 evaluated heterospermic pooled semen samples. VAP = average path velocity (mμ/s); VSL = straight-line velocity (mμ/s); VCL = curvilinear velocity (mμ/s); ALH = amplitude of lateral head displacement (mμ/s); BCF = beat cross-frequency (Hz); STR = straightness (%); LIN = linearity (%).*: significance at $P < 0.01$.

Table 3. Reproductive performances of rabbit does.

Cycle	1		2		3		4		5		6		Effects		
Group	**C**	**IV**	**C**	**IV**	**C**	**IV**	**C**	**IV**	**C**	**IV**	**C**	**IV**	**Group**	**Cycle**	**GxC**
Receptivity (%)	0.00 ± 0.00	0.00 ± 0.00	50.00[A] ± 8.33	100.00[B] ± 0.00	100.00 ± 0.00	100.00 ± 0.00	0.00[A] ± 0.00	100.00[B] ± 0.00	100.00 ± 0.00	100.00 ± 0.00	90.00[A] ± 9.09	100.00[B] ± 0.00	**	**	**
Fertility (%)	60.00 ± 0.00	60.00 ± 0.00	60.00[A] ± 0.00	100.00[B] ± 0.00	60.00[A] ± 0.00	50.00[B] ± 0.00	60.00[A] ± 0.00	100.00[B] ± 0.00	40.00[A] ± 0.00	80.00[B] ± 0.00	80.00 ± 0.00	80.00 ± 0.00	**	**	**
Live-born kits (N°)	3.30[a] ± 0.47	4.60[a] ± 1.30	5.60[b] ± 1.55	7.30[b] ± 0.42	5.20[a] ± 1.20	4.70[a] ± 1.32	8.30[b] ± 0.52	8.00[b] ± 0.42	2.60[a] ± 1.33	6.60[a] ± 0.85	5.70[b] ± 1.05	6.60[b] ± 1.16	NS	*	NS

20 does/group/cycle (20 x 6= 120 does/group). Values are expressed as mean ± SD. C= control group; IV= intravaginal group; Group= effect of Group; Cycle= effect of Cycle; GxC= interaction between Cycle and Group; *: P < 0.01; **: P < 0.001.

A, B: significant differences between groups (P < 0.001) for receptivity and fertility; a, b: significant differences between cycles (P < 0.01) for live born kits.

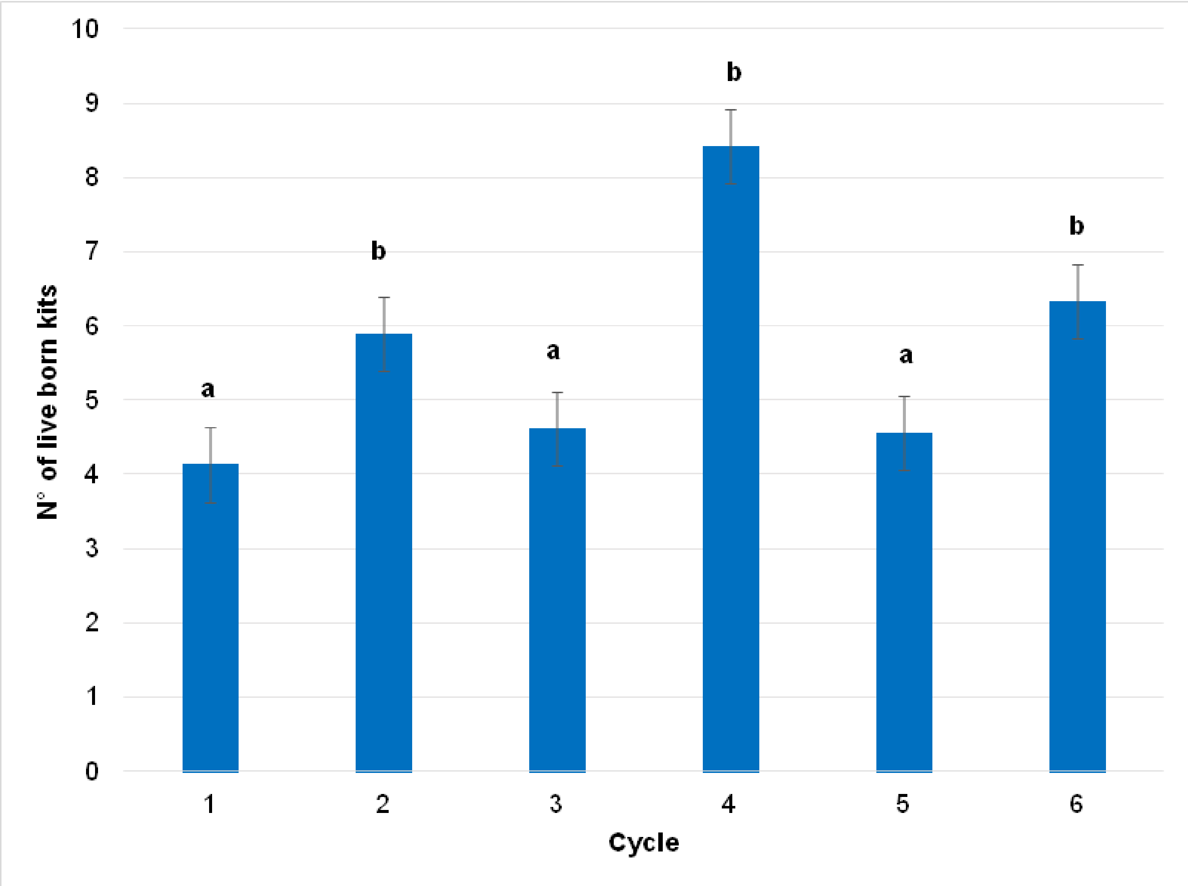


Figure 20. Effect of insemination cycle on live-born kits number. Values are expressed as mean ± SD; [a,b]: significant difference between different letters ($P < 0.01$).

Table 4. Correlation coefficients between sperm parameters and doe reproductive characteristics.

	VAP	VSL	VCL	ALH	BCF	STR	LIN	Total motility	Progressive motility	Live sperm	Sperm with intact acrosome	Fertility	Receptivity
VSL	0.908**												
VCL	0.082	−0.264**											
ALH	−0.628**	−0.863**	0.605**										
BCF	0.589**	0.841**	−0.483**	−0.934**									
STR	0.655**	0.907**	−0.521**	−0.943**	0.950**								
LIN	0.780**	0.955**	−0.522**	−0.944**	0.878**	0.950**							
Total motility	0.557**	0.510**	−0.044	−0.343**	0.260**	0.325**	0.433**						
Progressive motility	0.776**	0.871**	−0.291**	0.756**	0.718**	0.788**	0.838**	0.816**					
Live sperm	−0.127	−0.184	0.164	0.293	−0.278	−0.237	−0.231	−0.173	−0.174				
Sperm with intact acrosome	0.174	0.131	0.198	−0.032	−0.008	0.061	0.050	0.224	0.171	0.401*			
Fertility	−0.226**	−0.228**	−0.032	0.157*	−0.200**	−0.216**	−0.192*	−0.112	−0.195**	0.031	0.071		
Receptivity	−0.055	−0.021	−0.041	−0.035	0.034	0.006	−0.003	−0.010	−0.047	0.001	0.000	0.138	
Live kits	−0.072	−0.053	−0.067	0.023	0.035	−0.031	−0.043	−0.139	−0.094	−0.013	−0.220	0.282**	−0.051

Based on means of 54 evaluated heterospermic pooled semen samples, and results are expressed for both the C and IV groups. VAP = average path velocity (mμ/s); VSL = straight-line velocity (mμ/s); VCL = curvilinear velocity (mμ/s); ALH = amplitude of lateral head displacement (mμ/s); BCF = beat cross-frequency (Hz); STR = straightness (%); LIN = linearity (%); Total motility = total motile sperm (%); Progressive motility (%); Sperm with intact acrosome (%). * = P < 0.01.** = P < 0.001.

Table 5. Summary of selected multiple regression model for fertility (CONSTANT).

Parameters (CASA and RSA)	Coefficient (R^2=4.3%)	S.E. (11.66)	R^2 (%)	P
CONSTANT	127.583	91.125	-	-
VAP	−0.561	0.802	2.6	0.03
VSL	0.364	0.804	2.7	0.03
VCL	0.127	0.326	0	-
ALH	−2.628	3.081	1	-
BCF	−0.270	0.594	2	-
STR	−0.605	0.987	2	-
LIN	0.376	1.003	2	-
Total motility	0.127	0.200	0.7	-
Progressive motility	−0.244	0.301	2.3	0.04
Live sperm	−0.009	0.225	0	-
Sperm with intact acrosome	0.039	0.261	0	-

Significance of statistical model = $P < 0.05$. S.E. = standard error; R2 = the partial R2 for each parameter; P = significance of individual parameters; CASA = computer-assisted sperm analysis; RSA = morphological analysis; VAP = average path velocity (mμ/s); VSL = straight-line velocity (mμ/s); VCL = curvilinear velocity (mμ/s); ALH = amplitude of lateral head displacement (mμ/s); BCF = beat cross-frequency (Hz); STR = straightness (%); LIN = linearity (%); Total motility = total motile sperm (%); Progressive motility (%); Sperm with intact acrosome (%).

The results presented come from both the C and IV groups data.

5.2 Experiment 2: A multifactorial evaluation of different reproductive rhythms and housing systems for improving welfare in rabbit does

Reproductive and productive performances

All does were similar in weight, BCS, and sexual receptivity. The housing system (C vs. S) showed significant effects on the following parameters: fertility ($P<0.04$), number of live born kits ($P<0.02$), number of weaned kits ($P<0.04$), average kit weight at weaning ($P<0.04$) and pre-weaning mortality ($P<0.04$). The reproductive rhythm only showed a significant effect on fertility ($P<0.03$), number of live born kits ($P<0.02$) and the kits average weight ($P<0.03$). Interactions between housing and rhythm were never significant. In particular, fertility rates were higher in SE (100%), SA (92%), and CE (88%), compared to CI (69%), CA (69%) and SI (54%). The number of live kits was higher in the C cage compared to the S cage. In S cages, the SE does had the lowest live born values. The number of weaned kits was highest in the SI (8.50) and SA (9.50) groups, which resulted in higher weaned litters weight values (5321 and 6008 g, respectively, for SI and SA), and consequently, better individual kit weights (631 and 632 g, respectively, for SI and SA). The C group had worse pre-weaning mortality values than the S group (Table 6).

Table 6. Effects of housing system and reproductive rhythm on reproductive performances of doe (means ± SD).

Housing system		C			S			Significance of effects		
Rhythm		**I**	**A**	**E**	**I**	**A**	**E**	**H**	**R**	**H x R**
AI doe weight	g	4218 ± 67.62	4285 ± 86.30	4175 ± 80.03	4111 ± 166.29	4391 ± 186.95	4520 ± 123.09	NS	NS	NS
BCS	score	1.07 ± 0.06	1.74 ± 0.07	0.88 ±0.08	0.82 ± 0.18	2.08 ± 0.75	2.86 ± 0.11	NS	NS	NS
Receptivity	%	49 ± 0.06	48 ± 0.07	50 ± 0.07	46 ± 0.14	67 ± 0.17	15 ± 0.10	NS	NS	NS
Fertility	%	69 ± 0.06	69 ± 0.07	92 ± 0.05	54 ± 0.14	88 ± 0.13	100 ± 0.00	P<0.04	P<0.03	NS
Live born kits	N	9.12 ± 0.53	9.12 ± 0.60	9.31 ± 0.53	9.32 ± 0.85	9.78 ± 1.67	6.25 ± 1.82	P<0.02	P<0.02	NS
Dead born kits	N	0.47 ± 0.24	0.53 ± 0.27	0.20 ± 0.18	0.00 ± 0.00	1.71 ± 1.17	1.50 ± 1.05	NS	NS	NS
Weaned kits	N	6.90 ± 0.33	7.11 ± 1.46	7.51 ± 0.42	8.50 ± 0.50	9.50 ± 0.65	7.17 [a] ± 0.19	P<0.04	NS	NS
Weight of weaned litter	g	4075 ± 187.15	3968 ± 197.86	4055 ± 234.15	5321 ± 373.37	6008 ± 472.82	4169 ± 641.80	P<0.01	NS	NS
Average weaned kit weight	g	607 ± 22.38	553 ± 17.42	582 ± 15.50	631 ± 37.04	632 ± 21.57	587 ± 24.71	P<0.04	P<0.03	NS
Pre-weaning mortality	%	1.52 ± 0.09	1.46 ± 0.10	1.42 ± 0.08	1.16 ± 0.09	1.04 ± 0.19	1.13 ± 0.09	P<0.04	NS	NS

N = 75 (20 Combi does x 3 groups x 6 reproductive cycles + 15 Standard does x 3 groups x 6 reproductive cycles)

CI: Combi cage with Intensive rhythm; CA: Combi cage with Alternating rhythm; CE: Combi cage with Extensive rhythm; SI: Standard cage with Intensive rhythm; SA: Standard cage with Alternating rhythm; SE: Standard cage with Extensive rhythm; H: housing effect; R: rhythm effect; H x R: interaction; AI: artificial insemination; BCS: Body Condition Score; SD: standard deviation; NS: not significant.

The effects of the housing system and the reproductive rhythm on the indices of global productivity are shown in Table 7. The housing system, the rhythm, and interaction between them had significant effects (P<0.01) on all evaluated parameters, except that the interaction did not affect the number of kindlings/year/doe. Among the C groups, the extensive rhythm (CE) positively affected the productive performance in the number of rabbits sold/year/doe and the live weight of rabbits sold/year/doe (32.95 and 81.71 kg, respectively). However, it had less effect on the kindlings/year/doe (4.89), as a consequence of the greater kindling interval (74.62 days), due to the long time required for the extensive reproductive rhythm. The SI group had the highest number (40.80) and total weight (118.51 kg) of rabbits sold/year/doe, and the lowest production losses (4.35 kg) among all groups, but it also had the highest annual replacement of does (130%). In contrast, the more extensive housing and reproductive rhythm (CE) increased does' longevity; this group had the lowest incidence of animals replaced (20%). The SA group displayed intermediate values in terms of the number of rabbits/sold/year/doe (39) and the live weight of rabbits sold/year/doe (93.18 kg). The CI and CA groups showed intermediate values for production losses, the kindling interval, and the number of kindlings/year/doe.

Table 7. Effects of housing system and reproductive rhythm on indexes of global productivity (mean ± SD).

Housing system				C			S	Significance of effects		
Rhythm		**I**	**A**	**E**	**I**	**A**	**E**	**H**	**R**	**H x R**
Rabbits sold/year/doe	n	26.20 ± 0.21	25.60 ± 0.22	32.95 ± 0.29	40.80 ± 0.69	39.00± 0.65	17.20 ± 1.17	P<0.01	P<0.01	P<0.01
Live weight sold/year/doe	kg	77.97±12.06	74.20± 12.21	81.71± 16.92	118.51± 19.01	93.18±17.65	43.00 ±11.76	P<0.01	P<0.01	P<0.01
Production losses	kg	13.36 ± 6.21	13.36 ± 6.13	20.64 ± 7.49	4.35 ± 7.61	19.32± 8.92	19.36 ± 7.88	P<0.01	P<0.01	P<0.01
Kindling interval	day	69.29 ± 3.45	69.29 ± 3.47	74.62 ± 5.90	63.14 ± 2.96	77.08± 2.24	82 ± 2.65	P<0.01	P<0.01	P<0.01
Kindlings/year/doe	n	5.27 ± 0.90	5.27 ± 0.70	4.89 ± 0.80	5.78 ± 1.00	4.73 ± 0.02	4.45 ± 0.40	P<0.01	P<0.01	NS
Annual replacement of does	%	30	60	20	130	60	40	P<0.01	P<0.01	P<0.01

N = 75 (20 Combi does x 3 groups x 6 reproductive cycles + 15 Standard does x 3 groups x 6 reproductive cycles)

CI: Combi cage with Intensive rhythm; CA: Combi cage with Alternating rhythm; CE: Combi cage with Extensive rhythm; SI: Standard cage with Intensive rhythm; SA: Standard cage with Alternating rhythm; SE: Standard cage with Extensive rhythm; H: housing effect; R: rhythm effect; H x R: interaction; SD: standard deviation; NS: not significant.

Behavioural patterns

The effects of housing and rhythm on does' behaviours are presented in Table 8. The housing system affected the animal ethograms, regarding the following behaviours: crouching with ears down (P<0.03) and ears erect (P<0.04), lying down with ears down (P<0.03), standing up with eras down (P<0.02), self-grooming (P<0.01), feeding (P<0.001), drinking (P<0.002), biting and sniffing bars (P<0.01). No significant differences were found in the effects of different rhythms or interactions, except for sniffing bars (P<0.03).

Static activities were the most frequent behaviours observed for all does. S does had higher mean values for crouching with ears down (14.33 vs. 8.11% for S and C groups, respectively), and C does had higher mean values for crouching with erect ears in (26.36 vs. 21.17% for C and S groups, respectively). S does had higher mean values for lying down with ears down (6.17 vs. 1.88% for S and C groups, respectively). Standing up with ears down was only observed in S cages, and the intensive rhythm showed the highest value (7.21). Conversely, standing up with erect ears was observed only in C cages.

Rabbit movements were not affected by either the housing system or the rhythm. Jumping was only performed in C cages, due to the presence of the platform.

Compared to does in C cages, does in S cages displayed higher frequencies of self-grooming (10.99 vs. 6.99% for S and C, respectively), feeding (1.93 vs. 1.07% for S and C, respectively), drinking (1.95 vs. 0.71% for S and C, respectively), biting bars (2.71 vs. 0.29% for S and C, respectively), and sniffing bars (2.64 vs. 0.78% for S and C, respectively). The I rhythm increased the frequency of biting and sniffing cage bars.

Table 8. Effects of housing system and reproductive rhythm on behaviours (mean ± SD).

Housing system	C			S			Significance of effects		
Rhythm	**I**	**A**	**E**	**I**	**A**	**E**	**H**	**R**	**H x R**
Crouching with ears down	5.11 ± 2.62	5.88 ± 2.61	5.24 ± 1.77	4.33 ± 1.92	18.19 ± 6.21	20.47 ± 6.78	P<0.03	NS	NS
Staying with ears down	1.47 ± 0.61	2.48 ± 0.81	0.02 ± 0.10	0.00 ± 0.00	2.14 ± 0.78	3.12 ± 3.12	NS	NS	NS
Sitting up with ears down	2.52 ± 1.34	2.24 ± 0.82	0.27 ± 0.84	0.76 ± 0.76	1.00 ± 1.21	0.00 ± 0.00	NS	NS	NS
Lying down with ears down	1.97 ± 1.00	2.68 ± 1.14	0.99 ± 0.36	3.52 ± 2.31	9.30 ± 4.02	5.70 ± 3.44	P<0.03	NS	NS
Crouching with erect ears	27.23 ± 3.48	25.81 ± 3.81	26.04 ± 3.63	24.33 ± 7.33	13.53 ± 3.35	25.67 ± 5.26	P<0.04	NS	NS
Staying with erect ears	7.65 ± 1.45	7.19 ± 1.31	7.55 ± 1.23	6.09 ± 2.05	9.97 ± 3.62	7.41 ± 1.83	NS	NS	NS
Sitting up with erect ears	15.40 ± 2.18	14.10 ± 2.44	16.28 ± 1.92	7.25 ± 1.95	10.05 ± 3.91	8.28 ± 3.10	NS	NS	NS
Lying down with erect ears	2.01 ± 1.35	6.45 ± 1.61	4.74 ± 1.90	6.90 ± 3.01	12.52 ± 4.45	10.94 ± 4.83	NS	NS	NS
Standing up with erect ears	0.78 ± 0.41	0.23 ± 0.23	1.66 ± 0.76	0.00 ± 0.00	00.00 ± 00.00	00.00 ± 00.00	P<0.02	NS	NS
Standing up with ears down	00.00 ± 00.00	00.00 ± 00.00	00.00 ± 00.00	7.21 ± 2.07	2.71 ± 7.07	00.00 ± 00.00	P<0.02	NS	NS
Running	4.40 ± 0.85	4.02 ± 0.98	4.90 ± 0.90	5.00 ± 1.90	0.67 ± 0.67	3.45 ± 1.24	NS	NS	NS
Exploring	4.69 ± 1.01	3.83 ± 1.04	3.77 ± 1.04	5.13 ± 1.55	3.17 ± 1.82	0.50 ± 0.35	NS	NS	NS
Jumping	3.20 ± 0.69	3.00 ± 0.81	5.35 ± 1.21	00.00 ± 00.00	00.00 ± 00.00	00.00 ± 00.00	P< 0.001	NS	NS
Self- grooming	6.13 ± 1.34	8.57 ± 1.67	6.28 ± 1.38	12.38 ± 3.10	10.10 ± 2.44	10.51 ± 2.12	P<0.01	NS	NS
Feeding	0.62 ± 0.37	1.58 ± 0.60	1.01 ± 0.87	4.06 ± 1.13	00.00 ± 00.00	1.75 ± 1.23	P< 0.001	NS	NS
Drinking	0.89 ± 0.40	0.56 ± 0.32	0.68 ± 0.40	4.52 ± 2.08	1.32 ± 0.83	00.00 ± 00.00	P<0.002	NS	NS
Biting bars	0.41 ± 0.49	0.44 ± 0.33	0.02 ± 0.46	4.87 ± 2.08	2.70 ± 1.95	0.56 ± 0.38	P<0.01	NS	NS
Sniffing bars	1.23 ± 0.70	0.93 ± 0.39	0.18 ± 0.76	3.65 ± 1.35	2.63 ± 1.45	1.64 ± 0.82	P<0.01	P<0.03	NS

N = 75 (20 Combi does x 3 groups x 6 reproductive cycles + 15 Standard does x 3 groups x 6 reproductive cycles).

CI: Combi cage with Intensive rhythm; CA: Combi cage with Alternating rhythm; CE: Combi cage with Extensive rhythm; SI: Standard cage with Intensive rhythm; SA: Standard cage with Alternating rhythm; SE: Standard cage with Extensive rhythm; H: housing effect; R: rhythm effect; H x R: interaction; SD: standard deviation; NS: not significant

Maternal attitude and tonic immobility test

Maternal attitude was assessed in terms of nest quality, resulting not significant neither for the group effect, nor for the rhythm (Table 9).

The TI test results were not significantly different between groups. The housing, rhythm, and their interaction had no significant effects (Table 9).

Salivary corticosterone

The effects of housing and reproductive rhythm on CORT levels are shown in Table 9. The rhythm and interactions had no significant effects on CORT levels. However, the housing system affected CORT levels (P<0.007). CORT values were approximately 3-fold higher among does in C housing compared to does in S housing.

Table 9. Effects of housing system and reproductive rhythm on maternal attitude, tonic immobility test and salivary corticosterone level (mean value ± SD).

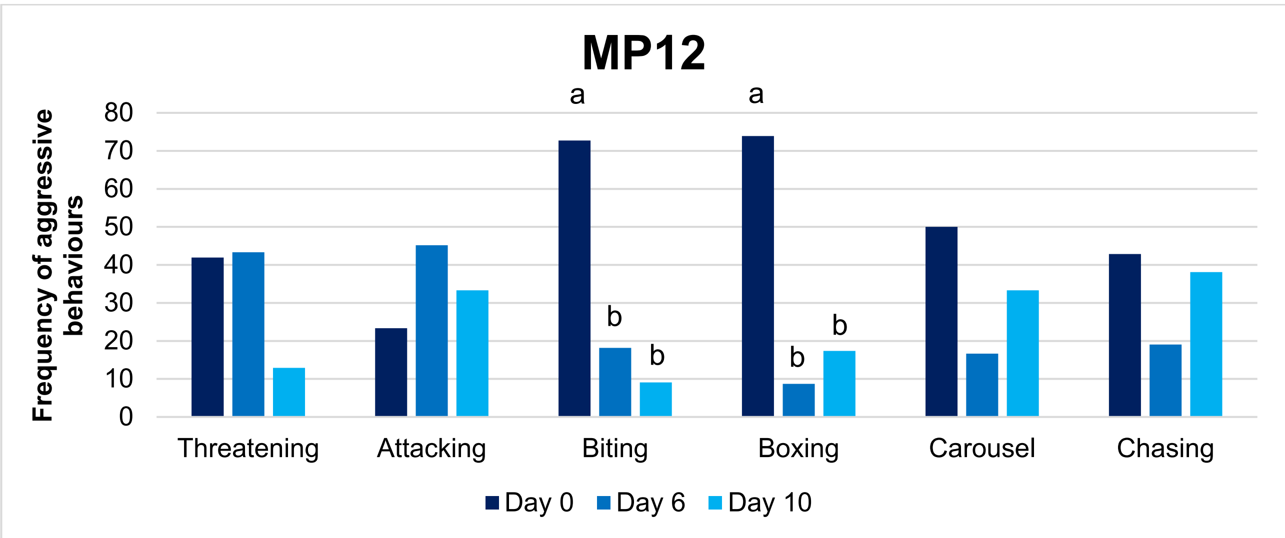
Housing system	C			S			Significance of effects		
Rhythm	**I**	**A**	**E**	**I**	**A**	**E**	**H**	**R**	**H x R**
Maternal attitude									
Nest mix	1.43 ± 0.08	1.55 ± 0.10	1.24 ± 0.07	1.38 ± 0.14	1.29 ± 0.18	1.44 ± 0.24	NS	NS	NS
Nest hair	1.80 ± 0.10	1.96 ± 0.12	1.84 ± 0.11	1.77 ± 0.23	1.57 ± 0.20	1.56 ± 0.24	NS	NS	NS
Nest material	1.59 ± 0.11	1.72 ± 0.12	1.45 ± 0.10	1.62 ± 0.21	1.43 ± 0.20	1.67 ± 0.29	NS	NS	NS
Tonic immobility (s)									
TI 1	6.17 ± 2.32	2.56 ± 1.80	10.96 ± 3.29	3.85 ± 3.85	9.10 ± 5.34	8.69 ± 5.24	NS	NS	NS
TI 2	4.94 ± 2.21	5.13 ± 2.18	9.59 ± 3.47	7.69 ± 5.33	9.10 ± 5.34	4.35 ± 3.42	NS	NS	NS
TI 3	3.70 ± 1.93	5.13 ± 2.18	5.53 ± 2.68	3.85 ± 3.85	4.54 ± 4.54	00.00 ± 00.00	NS	NS	NS
Salivary corticosterone (ng/mL)									
CORT	2856 ±173.68	2764 ±195.81	2929 ±118.19	1385 ±100.72	1036 ±228.03	1140 ±194.67	P<0.007	NS	NS

N = 75 (20 Combi does x 3 groups x 6 reproductive cycles + 15 Standard does x 3 groups x 6 reproductive cycles).
CI: Combi cage with Intensive rhythm; CA: Combi cage with Alternating rhythm; CE: Combi cage with Extensive rhythm; SI: Standard cage with Intensive rhythm; SA: Standard cage with Alternating rhythm; SE: Standard cage with Extensive rhythm; H: housing effect; R: rhythm effect; H x R: interaction; TI: tonic immobility; CORT; salivary corticosterone ; SD: standard deviation; NS: not significant.

5.3 Experiment 3: Effect of different management protocols for grouping does on aggression and dominance hierarchies

Considering MP12 and MP18, the frequency of aggressive interactions decreased noticeably from day 0 after regrouping, but no significant differences were detected between days 6 and 10 (Figure 21), while MP22 did not show significant differences when each interaction was considered individually. Analysing the number of aggressive acts, in the generalized linear model, protocols did not differ (MP: $F_{1,4}$ = 1.07, P = 0.36), whereas day and a day x protocol interaction were significant (Day: $F_{2,8}$ = 13.78, P = 0.003; Interaction: $F_{2,8}$ = 4.44, P = 0.05). Regarding the interaction, the variable day was significant only for MP18, but not for MP12 (MP12: $F_{2,8}$ = 1.28, P = 0.33; T18 $F_{2,8}$ = 25.01, P = 0.0004). When day 10 was deleted in order to include all MPs, they did not differ in the number of aggressive encounters (MP: $F_{2,6}$ = 1.96, P = 0.22; Interaction: $F_{2,6}$ = 4.66, P = 0.06), however days differed (Day: $F_{1,6}$ = 31.08, P = 0.001). The number of aggressive encounters decreased from day 0 to day 6 ($F_{1,22}$ = 11.58, P = 0.003, N = 30), but no effects of protocol ($F_{2,22}$ = 0.24, P = 0.79, N = 30) and trial ($F_{4,22}$ = 2.54, P = 0.06, N = 30) were found when all MPs were considered. Similarly, for MP12 and MP18, biting showed significant differences in respect of the day of observation ($F_{2,22}$ = 6.92, P = 0.005, N = 30), but protocol ($F_{1,22}$ = 0.2, P = 0.66, N = 30) and trial ($F_{4,22}$ = 1.47, P = 0.25, N = 30) did not.

A)



B)

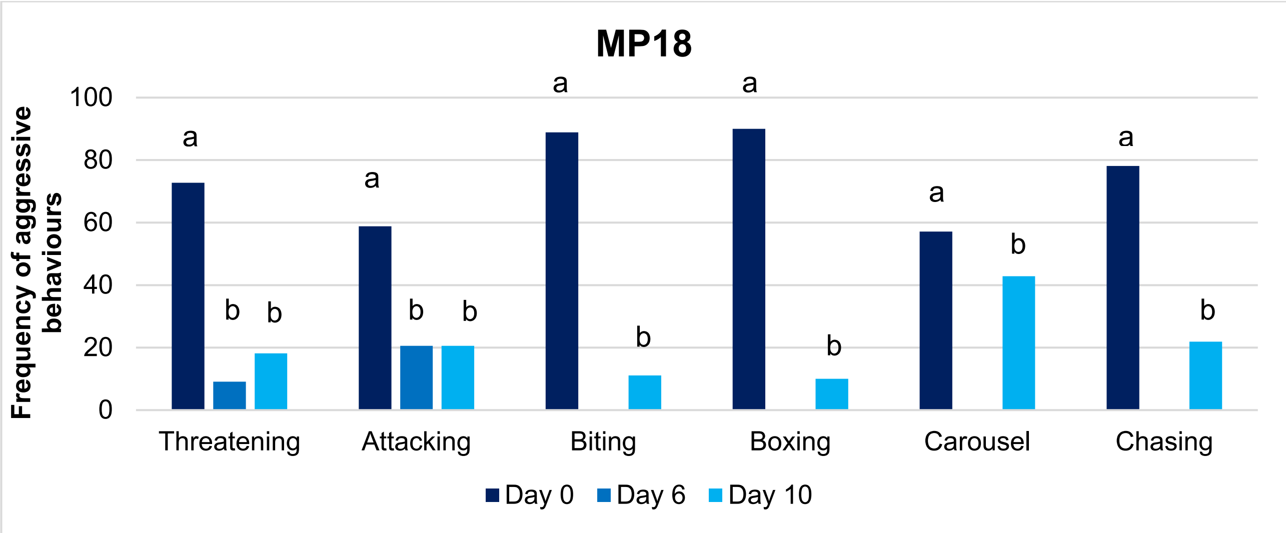


Figure 21. Frequency of aggressive interactions on days 0, 6 and 10 after regrouping in does MP12 (A) and MP18 (B). Threatening and attacking (as mild aggressions), biting and boxing (as severe aggressions), and carousel and chasing (as chasing behaviours) were combined for analyses. Different letters indicate $P < 0.05$.

When in all MPs the types of interactions were grouped, MP18 and MP22 showed more aggressive interactions classified as mild (threats and attacks), than MP12, on the day of regrouping (Table 10) (MP12 vs. MP18: $t_8 = -3.73$, $P_{adj.} = 0.045$; MP12 vs. MP22: $t_8 = -4.16$, $P_{adj.} = 0.026$). On day 6, MPs did not differ (all P-values above 0.39).

Table 10. Means and standard errors of mild (A) and severe (B) aggressive interactions on day 0 and 6 after regrouping for all MPs.

A)

Day	**MP12**	**MP18**	**MP22**
0	0.88^b (0.29)	2.14^a (0.17)	2.26^a (0.16)
6	1.33^a (0.26)	0.70^a (0.32)	1.49^a (0.24)

B)

Day	**MP12**	**MP18**	**MP22**
0	1.55^a (0.21)	1.16^a (0.25)	0.63^a (0.32)
6	-0.28^a (0.50)	-13.6^a (393.5)	0.28^a (0.38)

MP12 = separation for 12 days *postpartum* (*pp*); MP18 = separation for 18 days *pp*; MP22 = separation for 22 days *pp*. Mean values with different superscripts within the same rows differed significantly at $P < 0.05$.

Only 3 out of 40 aggressive interactions occurred between 04:00 and 08:00. Disregarding this time slot, the number of aggressive encounters increased with time from 20:00 to midnight ($F_{2,33}$ = 7.11, P = 0.003) with a linear (t_1 = 2.4, P = 0.02) and an exponential (square) term (t_1 = 2.5, P = 0.02) (Figure 22).

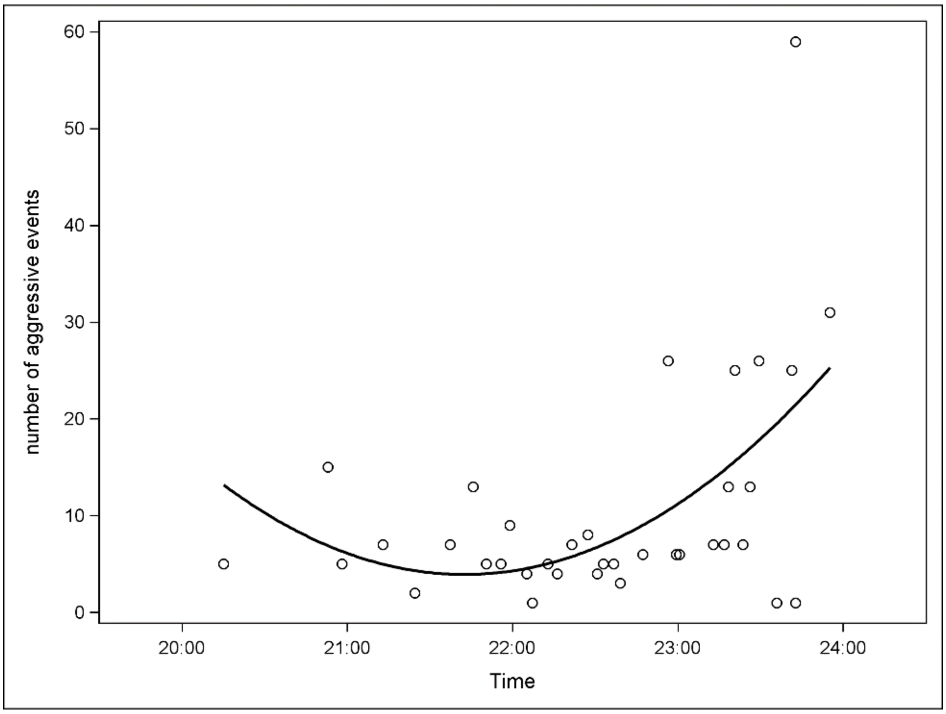


Figure 22. Number of aggressive interactions in does after grouping considering the time of day.

Dominance hierarchy

The stability coefficients were very high (mostly above 0.6) and did not differ among MPs ($F_{2,9}$ = 1.10, P = 0.38). However, trials differed ($F_{4,9}$ = 6.38, P<0.01) (Figure 23). In trial 2, the Elo-rating program did not generate a value of social stability for MP18 and MP22, possibly because the number of interactions was too low.

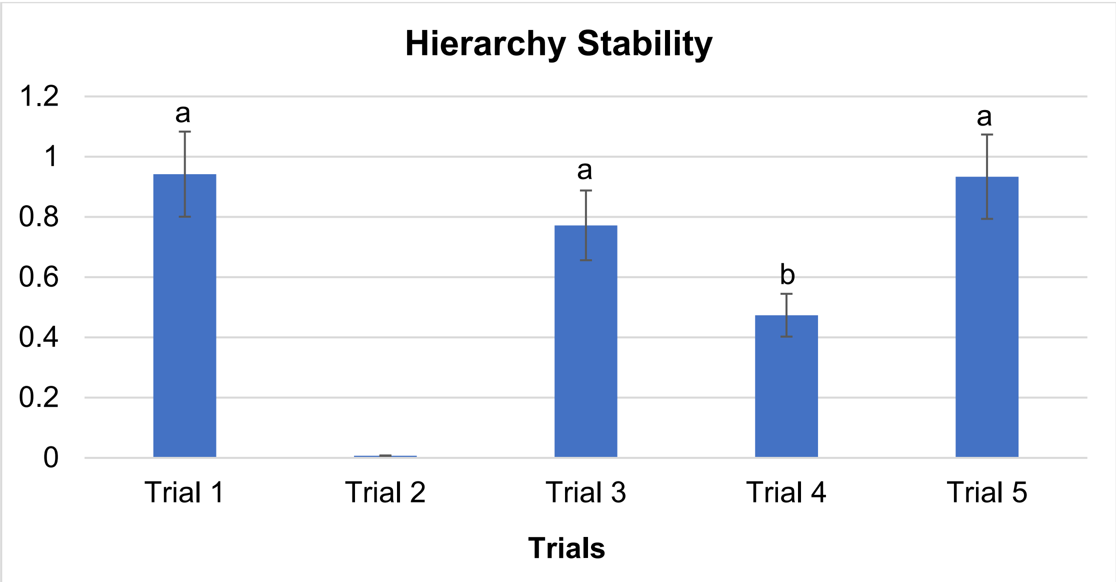


Figure 23. Hierarchy Stability Indices of the three MPs along consecutive trials. Different letters indicate $P < 0.05$.

6. DISCUSSIONS

6.1. Experiment 1: Effects of an intravaginal GnRH analogue administration on rabbit reproductive parameters and welfare

Our results indicated that the addition of GnRH to rabbit sperm did not significantly impair motility characteristics, similar to prior findings (Fernàndez et al., 2017). However, Gogol et al. (2014) reported that the addition of GnRH analogue on rabbit semen extended with Galap extender did not affect any of the sperm parameters studied. The main function of GnRH is to induce the release of gonadotrophic hormones, mainly LH, from the anterior pituitary gland and consequently controlling the gonadal activity (Sajjad, et al., 2007). It has been stated that gonadotropin are actively involved in male reproductive functions and stimulate the secretion of testosterone hormone required for spermatogenesis and sperm transport (Bearden and Fauquay, 1980).

Numerous proteolytic enzymes are present in the seminal plasma and spermatozoa of mammals and avian species (Métayer et al., 2002; Kotłowska et al., 2005), and GnRH analogues could be susceptible to peptidase degradation. Vicente et al. (2011) suggested that decreased hormonal activity is largely due to seminal plasma—finding that a low dilution rate of seminal plasma is associated with high amino peptidase activity, and that GnRH analogues can be hydrolyzed like many other proteins and peptides. In contrast, a high dilution rate is associated with low amino-peptidase activity, allowing the attainment of high ovulation frequency.

The intravaginal absorption of GnRH added to diluted semen may be influenced by both the mucosal state (secretions induced by the receptivity status) and the sperm concentration (Viudes de Castro, 2007). Sexual receptivity is an important factor in the pregnancy rate, as negative results have been obtained after insemination of non-receptive does (Armero et al., 1994). Good assessment of sexual receptivity in rabbit does is fundamental to successful AI, but is subjective and affected by technician experience (Viudes de Castro, 2007). In our study, does inseminated with diluted semen plus Dalmarelin showed equal or greater sexual receptivity compared to the control group, resulting in a higher or at least equal fertility rate throughout reproductive cycles. In contrast, the control group showed an improved fertility rate only in cycle 3. Our results are in agreement with those of Zapletal and Pavlik (2008) and Canali et al. (1991), who recorded reduced fertility after repeated intramuscular GnRH treatments due to high numbers of fetuses and a higher incidence of hemorrhagic follicles, respectively. In contrast, Dal Bosco et al. (2012) reported that the intramuscular GnRH administration was associated with a higher fertility rate (80%) compared to intravaginal GnRH administration (20%).

It has been suggested that a fraction of GnRH analogue can be lost due to seminal backflow, and that a reduced seminal dose can help reduce the required quantity of added hormone (Quintela et al., 2004). In our present study, the seminal dose volume was very low, possibly explaining the better results in the intravaginal group, which received a similar amount of hormone (0.3 mL/doe) as the control group (0.2 mL/doe). The worse performance of the control group may have also been influenced by anti-GnRH antibody formation due to the repeated intramuscular hormone application, as previously described by Canali et al. (1991). On the other hand, the level of anti-eCG antibodies is reportedly eCG dose dependent. Notably, the immune reaction appeared after cycle 6, and was not significantly correlated with the reproductive parameters (Theau-Clément et al., 2008). It is also possible that the difference between groups was amplified by the fact that intramuscular administration is probably the more stressful method of ovulation induction (Castellini, 2007). Furthermore, even the limited number of animals used for the experiment (10 does/IV and 10 does/C) may have influenced the results obtained.

The two groups in our study did not significantly differ with regards to the number of live-born kits, which is in agreement with the findings of a previous study by Quintela et al. (2004).

When assessing correlations between sperm parameters and fertility, receptivity, and number of live-born kits, several factors should be standardized, including the semen collection and dilution, and the doe's reproductive status. In our present study, we identified several significant correlations between sperm motility, morphological parameters, and reproductive aspects. We found a significant positive correlation between the two sperm morphological parameters: % of live sperm and % of sperm with intact acrosome. These results are in agreement with the report by Saacke and White (1972), who analyzed bull semen and showed a significant correlation between sperm fertility and the percentage of spermatozoa with intact acrosome after 2 h of incubation at 37°C. On the other hand, another study showed a higher percentage of sperm with intact acrosome among fertile stallions (74%) than infertile ones; however, these percentages are not often correlated with in vivo fertility. The percentage of sperm cells with normal morphological traits is an important indicator and is highly correlated with fertility rate (Zhang et al., 1990). Regarding motility, our present results revealed that progressive motility was significantly positively correlated with motility characteristics, including VAP, VSL, ALH, BCF, STR, and LIN. These results are in agreement with the findings of Nagy et al. (2015) who evaluate the different kinematic (velocity) parameters of frozen/thawed bull semen and found that VAP is the most useful semen motility characteristic that has clinical relevance in fertility prediction. Unfortunately, we observed a significant negative correlation between progressive motility and the does' fertility rate. In the literature, there is no clear evidence demonstrating that a positive correlation between sperm motility parameters and sperm fertility is good, and that it can truly reflect the reproductive parameters of the doe. This lack of information is commonly due to the variation between individual animals and the use of insemination doses with spermatozoa numbers that are too high or too low.

The inclusion of morphological and motility parameters in a multiple regression model to evaluate fertility explained only a very low portion of the variation. However, some sperm parameters (VAP, VSL, and total motility) had a significant impact. The low correlation obtained in the present trial is likely due to the low number of sperm parameters evaluated. It is difficult to find a perfect test for evaluating in vivo fertility, i.e., one that consistently shows high correlations between the parameters and the animals' fertility rate. An ideal sperm assay would evaluate several spermatozoa attributes in a large number of sperm, to determine the proportion of the cells that possess all of the characteristics necessary to fertilize the oocyte (Graham, 1996).

6.2 Experiment 2: A multifactorial evaluation of different reproductive rhythms and housing systems for improving welfare in rabbit does

In this study, reproductive performances regarding does' weight at artificial insemination, BCS, and sexual receptivity were not affected by the housing system or the reproductive rhythm.

A previous study by Theau-Clément et al. (2016) showed that the E rhythm improved the fertility rate among rabbit does. In that study, a less intensive rhythm led to the best results in fertility. In fact, inseminating after kit weaning could improve the percentage of pregnancies, due to the lack of hormonal antagonism between lactation and pregnancy. Moreover, according to Castellini et al. (2003), the Extensive rhythm compensated for the lower production efficiency, because doe longevity was improved compared to the I and A rhythms.

When an E rhythm was applied in S housing (SE), we observed the worst reproductive performance and the lowest global productivity indices. This apparently contradictory finding might be explained by the prolonged fertilization-conception interval combined with less space available for motor activities, compared to the E rhythm applied in larger cages (CE). The lack of space could have led to excessive increases in weight and BCS, and a consequent lower conception rate, as the reproductive career progressed (Cerolini et al., 2015). This hypothesis was supported by our findings among does in the CE group. This group showed higher motor activity, lower feeding rates (see jumping and feeding in following discussion), and increases in number of live kits and weaned kits, which resulted in better reproductive performance in the C cages.

In the S cage, compared to the I rhythm applied at 11 days postpartum, the A rhythm appeared to be more adapted to doe physiology. The A rhythm provided close to 90% fertility, which resulted in the highest live kits and weights of weaned litters and the lowest pre-weaning mortality, compared to all the other experimental groups. These findings were consistent with those reported in a previous study by Castellini et al. (2003), where the reproductive parameters improved with a semi-intensive reproductive rhythm (AI at 1 day postpartum alternating with AI performed after kits weaning). That rhythm reduced the energy deficit of females compared to a standard rhythm.

C cages showed different reproductive performances, but none of the three groups analysed achieved the productivity observed in the SI group. In fact, it should be pointed out that, in selecting genetic improvements for rabbit does, favourable maternal breeding characteristics were determined based on productivity in a barren environment (i.e. a standard cage) (Theau-Clément, 2007). Consequently, the animals exhibited high productivity in SI.

We also found that the housing system affected does' behaviours. Animals housed in C cages showed a broader behavioural repertoire, consistent with a study by Mugnai et al. (2009). Moreover, in accordance with a study by Gunn-Dore and Morton (1993), static activities were performed most frequently in all experimental groups. This probably resulted from the fact that all direct observations were recorded only during the light period, between 8.00 a.m. and 16.00 p.m., when these crepuscular animals are typically less active (Jilge and Hudson, 2001).

S animals showed higher frequencies of feeding and drinking than C animals. This result was consistent with results from Gunn-Dore (1997), where extreme boredom induced animals to eat greater amounts of food. Moreover, the higher frequencies we observed in self-grooming and stereotypical behaviours among S does were consistent with results from Mugnai et al. (2009), who compared standard to colony housing.

Stereotypical disorders are indicators of anxiety (Poderscek et al., 1991). These behaviours usually replace normal animal behaviours in situations where normal ones are inhibited when eliciting stimuli are lacking and space is insufficient (Gunn-Dore and Morton, 1993). In our study, the larger cage dimensions and the use of enriched material, such as the platform in the C system, led to distraction from abnormal behaviours. This result was consistent with the study by Trocino et al. (2019), which showed that the presence of an elevated platform allowed rabbits to move more, to rest in a more comfortable position, and to increase exploratory behaviour. Additionally, Hansen and Berthelsen (2000) demonstrated that rabbit does in standard cages became bored more frequently, showed higher comfort activities, and spent more time biting the bars compared to does reared in innovative cages with an elevated platform. Moreover, jumping was observed only in C cages, due to the greater space availability; in S cages, jumping was not possible, due to the small cage dimensions.

Morton et al. (1993) showed that high cages (75 cm) were fundamental for allowing rabbits to sit upright without the ears touching the top of the cage. In our study, does in S cages more frequently stood up with the ears down, due to insufficient cage height (35 cm), compared to does in C cages. The C cage height (113 cm) allowed standing up with erect ears. However, the relevance of this behaviour as an indicator of animal welfare might be questionable in commercial farm conditions, due to the lack of predators (Princz et al., 2008).

The TI test did not show any significant differences among groups. This result might be explained by the frequent manipulation of rabbits by the farmer during routine management. This finding was consistent with several previous studies that showed that the state of fear strongly declined when animals became accustomed to human presence and contact. Low TI scores improved the general welfare of does, with positive effects on reproduction performance and health status (Kersten et al., 1989; Jiezierski and Konecka, 1996).

The salivary CORT concentrations were affected by the housing system, with higher CORT values in C does. This result was inconsistent with some previous studies (Koolhaas et al., 1993; Koob and Heinrichs, 1999; Cornale et al., 2016). This discrepancy might be explained by the greater amount of stimuli in the innovative C system. In brief, the greater space available in C housing could have allowed greater body exposure, due to the lower stocking density. This feeling of exposure might have led breeding does to react with an anti-predator reaction (physiological stress response), feeling more vulnerable in the large cage compared to the smaller conventional cages (Monclus et al., 2006). These effects might explain the elevated CORT levels observed in C does. Another potential explanation might be that exercise, like jumping, in our study, might have acted as a stimulus for the hypothalamus-pituitary-adrenal (HPA) axis, which would result in significant increases in circulating cortisol levels (Hill et al., 2008). Significant differences in this exercise response would only be observed by comparing sedentary individuals (S does) to highly trained individuals (like C does). In C does, the activation of the HPA axis and the activation of the sympathetic-adrenomedullary system might have contributed to mobilize the energy necessary to cope with the environmental stimuli (Boissy, 1995; Holst, 1998). These conditions might have had negative consequences on reproductive requirements, as we observed in does allocated to the I or A rhythms. Despite this, in accordance with our study, Pérez-Fuentes et al. (2020) recently showed greater levels of cortisol in rabbits does placed in collective systems compared to standard housing, which may suggest that they were housed under worse welfare conditions. The lower CORT levels observed in the S group might be explained by the so called 'coping response'. This response refers to any behaviour that apparently attenuates stressor-induced physiological responses (Rushen and Mason, 2006), due to the calming effect (Mason and Latham, 2004). Compared to C groups, the S groups displayed more stereotypical behaviours (biting and sniffing cage bars), which might have induced a calming effect, and consequently, maintained normal CORT levels.

Furthermore, it must be taken into account that other factors may also have influenced the results, such as cleanliness of the cage, state of health of the doe, stress in handling in control groups. In conclusion, further studies need to be performed to understand properly if the new type of cage proposed really matches animals' physiological needs.

6.3 Experiment 3: Effect of different management protocols for grouping does on aggression and dominance hierarchies

The aim of the present study was to investigate whether aggressive interactions of breeding does can be reduced by keeping them isolated for more than 12 days after parturition. Being gregarious animals, it would be desirable to keep rabbit does in groups for ethological reasons, but this results in social conflicts with increase of stress and injuries, reducing their welfare and performance (Rommers et al., 2006; Mugnai et al., 2009; Andrist et al., 2012). In addition, there can be aggression towards offspring. As found by Mykytowycz and Dudzinski (1972), does tolerate their own kits, but attack kits from other does. In this respect, Szendrő and McNitt (2012) recorded a high frequency of bitten and injured litters by competitive does, reducing the chance of survival with lower productive performances and less income for the farmer. However, Albonetti and Farabollini (1994) found a large decrease in terms of aggressive interactions after the establishment of a hierarchy and suggested that social interactions between rabbit does are mostly friendly over time, after a first period of fights.

In our study, for MP12 and MP18, the frequency of different categories of aggressive interactions were higher on the day of regrouping, with a dramatic decrease after 6 days. In contrast, almost no change was found between 6 and 10 days suggesting that aggressive interactions ceased after the social rank was established (Albonetti et al., 1990b; Andrist et al., 2013). It has generally been observed that regrouping unfamiliar animals leads to an increase in aggressive behaviours at the moment of group formation (Mykytowycz, 1958; Albonetti et al., 1990a) because a new dominance hierarchy needs to be established (dominance aggression), and also probably to compete for resources and space (territorial aggression) (Mykytowycz, 1958; Graf et al., 2011).

Only the factor number of days after parturition clearly affected the frequency of biting as the most damaging aggressive interaction, with a high frequency of occurrence after regrouping and a subsequent decrease in the course of the six-day observation period. Biting is considered a serious interaction due to the potential for severe injury. Our findings confirm other studies (Mykytowycz, 1958; Lehmann, 1991) showing that, although aggressive chasing and submissive retreat remain common, overt fighting becomes rare after the order of dominance has been established if the group composition remains intact. This suggests that most of the agonistic encounters were caused by dominance aggression because a new hierarchy needed to be established after the animals were grouped, also caused by protection towards the litter (Szendro and McNitt, 2012). In case of territorial aggression agonistic encounters would remain frequent (Mykytowycz, 1958). Moreover, Larsen and Grattan (2012) found that in mice prolactin induces neurogenesis in the female with critical changes in the mood and behavior in the *postpartum* period. It is well known that prolactin has a complex role in regulating aspects of maternal behaviour (Gonzà et al., 1996), therefore can be probably involved in aggressions, aimed at protecting the kits. When correlating hormonal regulation of maternal behaviour with lactation curve of rabbit does, there seems to be a link between the timing of aggressions and milk production, as previously reported by Zomeño et al. (2018). In fact, in our study, MP12 showed the highest frequency in severe aggressive interactions, probably linked to the highest milk output, respect MP18 and MP22 that showed more mild aggressive interactions, probably due to the descent phase of the lactation curve. In rabbits the curve of lactation is asymmetric with a convex ascending and a concave descending period after the peak of lactation on day 18-19 after parturition (Lebas, 1968).

However, when considering all types of aggression, fewer severe aggressive interactions were present in MP18 than in MP12. In particular, MP18 and MP22 showed more mild aggression without body contact than MP12 on the day of regrouping. Therefore, the level of aggression seemed to be affected by the durations of separation between does, following parturition, probably due to the greater age of kits that were, after a longer isolation, less vulnerable and so does don’t need to apply severe aggressive interactions like biting or boxing to protect them.

The change in the frequency of biting between 6 and 10 days after regrouping was not significantly different between protocols, suggesting that a hierarchy was established within a few days and biting declined rapidly.

Aggressive interactions were most frequent in the dark hours following regrouping and no systematic observations were carried out between the grouping in the morning and 20.00. Since the trials were spread from late summer to spring the relationship between time-of-day and amount of aggressive interactions is difficult to interpret, but the highest frequency was found close to midnight when it was always dark. This can be a problem for farm management because the farmer might not be aware of the aggressive behaviours.

Dominance hierarchy

When animals form social groups, it is possible to determine their order within the dominance hierarchy (Hinde, 1976). According to Elo (1978), individuals with similar Elo-ratings (and thus competitive abilities) may be considered to belong to the same category or class, while dissimilar Elo-ratings are predictive of clear dyadic dominance relationships. To the best of the authors' knowledge, there have not been any studies on the strength or stability of hierarchies in breeding rabbit does before. The observed stabilities were higher than 0.6 in most trials, which indicates stable hierarchies (McDonald and Shizuka, 2013). Moreover, our data displayed a strong effect of trial period which might be due to season since the first trial was carried out in September, the third and fourth in the winter and the fifth in February/March. It appears that the groups outside the natural breeding season, namely in the winter, had lower stabilities. Stability might result from higher aggressive interactions due to a higher level of testosterone, confirmed by Birganti et al. (2003) where an increased testosterone induced agonistic interactions in dominant rabbits. Does had smaller anogenital distances in the winter trials which supports this interpretation (Michèle Braconnier, personal communication).

7. GENERAL CONCLUSIONS

Under intensive farming conditions, rabbit does are currently house individually in cages that could not satisfy their behavioural patterns due to the small size. The lack of enough space contribute to stereotypes appearance due to reduction of animals' welfare. Moreover, the most common insemination technique is based on GnRH intramuscularly injected to induce ovulation, followed by insemination 11 days *postpartum*: this intensive rhythm requires high yearly replacement of animals that cannot sustain the enormous energy demand.

In view of the ongoing effort to move to an animal-friendly rearing system, my PhD project aimed at evaluating alternative reproductive managements and housing systems to contribute improving the sector, introducing also less invasive and stressful practices, more respectful of the physiology and welfare of rabbit does.

Regarding my first trial aimed at reducing the stressful technique of inducing ovulation through intramuscular GnRH injection, findings indicate that the incorporation of GnRH in a seminal dose could be used for ovulation induction in rabbit does. This method achieved sexual receptivity, fertility, and number of live-born kits equal or greater to those with conventional GnRH administration. Intravaginal administration may also carry lower risks of haemorrhagic follicles and thereby enable a higher conception rate and a longer reproductive career for the doe.

Further studies are needed to determine the ideal GnRH level for intravaginal administration based on the doe's physiological status. Compared to the intramuscular absorption capacity, the intravaginal absorption capacity is about 10 times smaller, such that this administration route requires a greater dosage. Identifying the optimal dose will enable avoidance of the conventional invasive treatment with GnRH analogues, along with the associated antibody formation and traumatic action, thus improving does physiological welfare and longevity.

In order to propose alternative housing systems to farmers, the standard cage used for rabbit does was compared with an innovative system, Combi cage, analysing their effects in combination with three different reproductive rhythms (Intensive, Alternating and Extensive), on behaviour, physiological traits and reproductive performances. We concluded that:

- the extensive rhythm was the best reproductive protocol in Combi cages. It avoided hormonal antagonism between lactation and pregnancy, which permitted does to restore energy to levels that could sustain reproductive activity. Moreover, the greater room for movement in Combi cages negatively affected reproductive performance among does allocated to the more intensive rhythms (I and A).
- in standard cages, the Alternating rhythm was the best choice for reproductive performance, because it allowed does to restore energy levels, obtaining the best results among the three different rhythms. In fact, even if performances were slightly lower than intensive rhythm, the annual replacement of does was less than 50%.
- ethologically, the Combi cage provided a wider ethogram, and does exhibited stereotyped

behaviours less frequently than does in standard cages.

- although stereotypical behaviour is considered a welfare indicator, in our study, stereotypical behaviours did not affect productivity in the standard system with Intensive rhythm.
- physiologically, corticosterone levels resulted to be greater in Combi does, as described by some recent studies.

We addressed how different farming factors (cage dimensions and reproductive rhythm) might affect rabbit does welfare and productivity. Practices considered key factors in rabbit welfare must provide greater productivity to facilitate producer adoption. Indeed, when practices that enhance animal welfare fail to lead to economic gains, changes will only occur when required ethically, because they will not improve rabbit or farmer welfare. Based on this, further studies are required to gain a deeper understanding of the relationships between animals, the environment and farming practices.

In recent years, efforts have also been made to introduce “part-time group housing” systems to reflect the social behaviour of the species, where rabbit does are kept separately and then reunited for a certain period of time. However, this system has not yet been successful because when animals are put together a new hierarchy has to be established, resulting in agonistic interactions with consequent injuries. In this direction, I decided to spent my period abroad in Switzerland where I investigated the development of hierarchies after the group formation, comparing two longer separation management protocols with the conventional 12 days after parturition normally applied on Swiss farms. Results indicated that a longer separation for 18 and 22 days did not reduce the number of total aggressive interactions, but a longer separation than 12 days was important to reduce severe behaviours between does. Additionally, the time point of 6 days after regrouping resulted to be crucial for the reduction of total aggressive interactions, as time frame necessary to establish the hierarchy. Maybe it would be better to give access to the other does gradually e.g. grouping them during the daylight with separation during the night, considering that fights usually occur during dark hours being crepuscular animals and so more active at night, thus having a gradual approach to regrouping the animals.

Moreover, to develop a suitable protocol for does regrouping after parturition, and also to give important suggestion for housing systems in terms of welfare, further investigations (physiological evaluations) should be performed to complement the hierarchy stability measurements, analyzing the possible correlations between milk production (lactation curve), associated hormonal changes and maternal behaviour (kits protection).

Therefore, much still needs to be done to bring the farmed rabbit to be a protected species in terms of welfare and physiology of the species, given that there is currently no EU legislation but only local guidelines.

8. REFERENCES

Albonetti, M.E., Dessi-Fulgheri, F., Farabollini, F., 1990a. Organization of behavior in unfamiliar female rabbits. Aggress. Behav. 17, 171–178.

Albonetti, M.E., Dessi-Fulgheri, F., Farabollini, F., 1990b. Intrafemale agonistic interactions in the domestic rabbit (*Oryctolagus cuniculus L.*). Aggress. Behav. 16, 77–86.

Albonetti, M.E., Farabollini, F., 1994. Social stress by repeated defeat: effects on social behaviour and emotionality. Behav. Brain Res. 62, 187–193.

Ajuogu, N.P.K., Nyeche, V.N., 2015. The Assessment of Artificial Breeding Pattern on Fertility in Rabbit Does In the Humid Tropics. IOSR-JAVS 8 (3), 81-85.

Andrist, C.A., Bigler, L.M., Würbel, H., Roth, B.A., 2012. Effects of group stability on aggression, stress and injuries in breeding rabbits. Appl. Anim. Behav. Sci. 142 (3-4), 182–188.

Andrist, C.A., van den Borne Bart, H. P., Bigler, L.M., Buchwalder, T., Roth, B.A., 2013. Epidemio-logic survey in Swiss group-housed breeding rabbits: Extent of lesions and potential risk factors. Prev. Vet. Med. 108, 218–224.

Andrist, C., Bigler, L., Würbel, H., Roth, B., 2014. Masking odour when regrouping rabbit does: effect on aggression, stress and lesions. Livest. Sci. 170, 150–157.

Armero, E., Garcia-Ximenez, F., Vicente, J.S., Baselga, M., 1994. Cycle synchronization of rabbit does naturally mated or artificially inseminated. World Rabbit Sci. 2, 107-113.

Bearden, H.J., Fauquay, J.W., 1980. Appl. Anim. Reprod. Prentice Hall, New Jersey, USA., pp.163

Bennet, A., Hayssen, V., 2010. Measuring cortisol in hair and saliva from dogs: coat color and pigment differences. Domest. Anim. Endocrinol. 39, 171-180.

Berger, H., Heinrich, N., Albrecht, E., Kertscher, U., Oehlke, J., Bienert, M., Schafe, H., Baeger, I., Mehlis, B., 1991. Gonadotropin-releasing hormone analogs: relationship between their structure, proteolytic inactivation and pharmacokinetics in rats. Regul. Pept. 33, 299-311.

Birganti, F., Della Seta, D., Fontani, G., Lodi, L., Lupo, C., 2003. Behavioural effects of testosterone in relation to social rank in the male rabbit. Aggress. Behav. 29, 269–278.

Blaszczyk, M., Slanina, T., Massanyi, P., Stawarz, R., 2013. Semen quality assessment of New Zealand white rabbit bucks. J. Microbiol. Biotechnol. Food Sci. 2, 1365-1376.

Blumetto, O., Olivas, I., Torres, A.G., Villagrà, A., 2010. Use of straw and wood shavings as nest material in primiparous does. World Rabbit Sci. 18, 237–242.

Boccia, L, Di Palo, R., De Rosa, A., Attanasio, L., Mariotti, E., Gasparrini, B., 2007. Evaluation of buffalo semen by Trypan blue/Giemsa staining and related fertility in vitro. Ital. J. Anim. Sci. 6, 739-742.

Boissy, A., 1995. Fear and fearfulness in animals. Q. Rev. Biol. 70, 165–191.

Boiti, C., 2005. Guidelines for the handling of rabbit bucks semen. World Rabbit Sci. 13, 71-91.

Bonanno, A., Mazza, F., Di Grigioli, A., Alicata, M.L., 2008. Body condition score and related productive responses in rabbit does. In: Proceedings of the 9th World Rabbit Congress, June 10–13, Verona, Italy.

Bornett, H.L.I., Morgan, C.A., Lawrence, A.B., Mann, J., 2000. The effect of group housing on feeding patterns and social behaviour of previously individually housed growing pigs. Appl. Anim. Behav. Sci. 70, 127–141.

Broom, D.M., 1986. Indicators of poor welfare. Br. Vet. J. 142, 524-526.

Broom, D.M., 1988. The scientific assessment of animal welfare. Appl. Anim. Behav. Sci. 20, 5-19.

Brun, J.M., Theau-Clément, M., Bolet, G., 2002. The relationship between rabbit semen characteristics and reproductive performance after insemination. Anim. Reprod. Sci. 70, 139–149.

Buijs, S., Keeling, L.J., Rettenbacher, S., Maertens, L., Tuyttens, F.A.M., 2011. Glucocorticoid metabolites in rabbit faeces – Influence of environmental enrichment and cage size. Phys. Behav. 104, 469-473.

Buijs, S., Tuyttens, F.A.M., 2015. Evaluating the effect of semi-group housing of rabbit does on their offspring's fearfulness: Can we use the open-field test? Appl. Anim. Behav. Sci. 162, 58-66.

Buijs, S., Maertens, L., Hermans, K., Vangeyte, J., Tuyttens, F.A.M., 2015. Behaviour, wounds, weight loss and adrenal weight of rabbit does as affected by semi-group housing. Appl. Anim. Behav. Sci. 172, 44–51.

Canali, C., Boiti, C., Castellini, C., Zampini, D., 1991. Risposta anticorpale delle coniglie trattate ripetutamente con PMSG nella pratica della sincronizzazione degli estri. 2° Meeting Nazionale Studio della Efficienza Riproduttiva degli Animali di Interesse Zootecnico. 24 Nov; 103-108. Italia.

Carenzi, C., Verga, M., 2009. Animal welfare: review of the scientific concept and definition. Ital. J. Anim. Sci. 8, 21-30.

Castellini, C., Dal Bosco, A., Mugnai, C., 2003. Comparison of different reproductive protocols for rabbit doe: effect of litter size and re-mating interval. Livest. Prod. Sci. 83, 131–139.

Castellini, C., Dal Bosco, A., Cardinali, R., 2005. Long term effect of post-weaning rhythm on the body fat and performance of rabbit does. Reprod. Nutr. Dev. 46, 195–204.

Castellini, C., 2007. Reproductive activity and welfare of rabbit does. Ital. J. Anim. Sci. 6 (1), 743-747.

Castellini, C., Lattaioli, P., 1999. Effect of number of motile sperms inseminated on reproductive performance of rabbit does. Anim. Reprod. Sci. 57, 111–120.

Cerolini, S., Marzoni Fecia di Cossato, M., Romboli, I., Schiavone, A., Zaniboni, L., 2015. Avicoltura e coniglicoltura. Point Veterinaire Italie 17-20, 395-470.

Cohen, J., 1960. A coefficient of agreement for nominal scales. Educ. Psychol. Meas. 20, 37–46.

Combes, S., Gidenne, T., Cauquil, L., Bouchez, O., Fortun-Lamothe, L., 2014. Coprophagous behavior of rabbit pups affects implantation of cecal microbiota and health status. J. Anim. Sci. 92, 652-665.

Conficoni, D., Cullere, M., Lago, N., Alberghini, L., Rossin, T., Dalle Zotte, A., Giaccone, V., 2020. Prevalence of post mortem lesions recorded in the largest Italian rabbit slaughterhouse over a fifteen-years period (2003-2017). World Rabbit Sci. 28, 39-47.

Conn, P.M., Crowley, W.F., 1991. Gonadotropin-releasing hormone and its analogues. N. Engl. J. Med. 324, 93-103.

Cornale, P., Macchi, E., Renna, M., Prola, L., Perona, G., Mimosi, A., 2016. Effect of cage type on fecal corticosterone concentration in buck rabbits during the reproductive cycle. J. Appl. Anim. Welf. Sci. 19, 90-96.

Curtis, S.E., 1987. Animal well-being and animal care. Vet. Clin. North Am. Food A. 3(2), 369-382.

Dal Bosco, A., Castellini, C., Mugnai, C., 2002. Rearing rabbis on a wire net floor or straw litter: behavior, growth and meat qualitative traits. Livest. Prod. Sci. 75, 149-156.

Dal Bosco, A., Rebollar, P.G., Boiti, C., Zerani, M., Castellini, C., 2011. Ovulation induction in rabbit does: Current knowledge and perspectives. Anim. Reprod. Sci. 129 (3-4), 106-117.

Dal Bosco, A., Cardinali, R., Rebollar, P.G., Millan, P., Brecchia, G., Castellini, C., 2012. Ovulation induction in rabbit does submitted to artificial insemination by adding lecirelin to the seminal dose. Preliminary results. In: Proc. 10th World Rabbit Congress, vol. 6; pp. 389-393.

Dal Bosco, A., Cardinali, R., Brecchia, G., Rebollar, P.G., Fatnassi, M., Millan, P., Mattioli, S, Castellini, C., 2014. Induction of ovulation in rabbits by adding Lecirelin to the seminal dose: in vitro and in vivo effects of different excipients. Anim. Reprod. Sci. 150, 44-49.

Dalle Zotte, A., Paci, G., 2013. Influence of rabbit sire genetic origin, season of birth and parity order on doe and litter performance in an organic production system. Asian-australas. J. Anim. Sci. 26 (1), 43-49.

Dantzer, R., Mormède, P., Henry, J.P., 1983. Physiological assessment of adaptation in farm animals. In: Farm Animal Housing and Welfare pp. 8-19. The Hague: Martinus Nijhoff.

Dresher, B., 1996. Deformations of vertebral column in breeding rabbits. In: Proceedings of the 6th World Rabbit Congress, Toulouse, France, pp. 417-421.

EFSA (European Food and Safety Authority), 2005. Scientific report "The impact of the current housing and husbandry systems on the health and welfare of farmed domestic rabbit". The EFSA Journal 267, 1-31.

EFSA (European Food and Safety Authority), 2011. Scientific Opinion "Scientific opinion concerning the welfare of animals during transport". EFSA Journal 9(1), 1966.

EFSA (European Food and Safety Authority), 2020. Scientific Opinion "Health and welfare of rabbits farmed in different production systems". EFSA Journal 18(1), 5944.

Elo, A., 1978. The rating of cheesplayers, past and present. New York: Arco.

Elwan, H.A.M., Elnesr, S.S., Mohany, M., Al-Rejaie, S.S., 2019. The effects of dietary tomato powder (Solanum lycopersicum L.) supplementation on the haematological, immunological, serum biochemical and antioxidant parameters of growing rabbits. J. Anim. Physiol. Anim. Nutr. (Berl) 103 (2), 534-546.

Erhard, W., Mendl, M., 1999. Tonic immobility and emergence time in pigs-more evidence for behavioural strategies. Appl. Anim. Behav. Sci. 61, 227-237.

Farm Welfare Animal Council, 1991. First Press Notice. 5/12 MAFF, London, UK.

Farm Welfare Animal Council, 2011. Ive freedoms. Available on http://www.fawc.org.uk/freedoms.htm. Visited on October 2019

Fernández Serrano, P., Casares Crespo, L., Viudes de Castro, M.P., 2017. Chitosan–dextran sulphate nanoparticles for GnRH release in rabbit insemination extenders. Reprod. Domest. Anim. 52, 72–77.

Ferrante, V., Verga, M., Canali, E., Mattiello, S., 1992. Rabbits kept in cages and in floor pens: reaction in the open-field test. J. Appl. Rabbit Res. 15, 700-707.

Forkman, B., Boissy, A., Meunier-Salaun, M.C., Canali, E., Jones, R.B., 2007. A critical review of fear tests used for cattle, pigs, sheep, poultry and horses. Physiol. Behav. 92, 340-374.

Fortun-Lamothe, L., 2006. Energy balance and reproductive performance in rabbit does. Anim. Reprod. Sci. 93 (1-2), 1-15.

Fortun-Lamothe, L., Combes, S., Gidenne, T., 2010. Contribution of intensive rabbit breeding to sustainable development. A semi-quantitative analysis of the production in France. World Rabbit Sci. 17 (2), 79-85.

Gamberini, A., 2001. Coniglicoltura. Edagricole, pp.192.

Gogol, P., Trzcińska, M., Bryła, M., 2014. Motility, mitochondrial membrane potential and ATP content of rabbit spermatozoa stored in extender supplemented with GnRH analogue [des-Gly10, D-Ala6]-LH-RH ethylamide. Pol. J. Vet. Sci. 17(4), 571–575.

Gonzà, G., Mariscal, I., Rosenblatt, J.S., 1996. Maternal behavior in rabbits: a historical and multidisciplinary perspective. Adv. Study Behav. 25, 330–360.

Graf, S., Bigler, L., Failing, K., Würbel, H., Buchwalder, T., 2011. Regrouping rabbit does in a familiar or novel pen: Effects on agonistic behaviour, injuries and core body temperature. Appl. Anim. Behav. Sci. 135 (1-2), 121–127.

Graham, J.K., 1996. Analysis of stallion semen and its relation to fertility. Vet. Clin. North Am. Equine Pract.12, 119-130.

Grasso, F., Napolitano, F., De Rosa, G., Quarantelli, T., Serpe, L., Bordi, A., 1999. Effect of pen size on behavioral, endocrine, and immune responses of water buffalo (Bubalus bubalis) calves. J. Anim. Sci. 77, 2039-2046.

Gunn-Dore, D., Morton, D.B., 1993. The behaviour of single-caged and group-housed laboratory rabbits. In: Proc. Fifth Fed. Europ. Lab. Anim. Sci. Ass. 80–84.

Gunn-Dore, D., 1997. Comfortable quarters for laboratory rabbits. In: Comfortable Quarters for Laboratory Animals, Eighth Edition Reinhardt V (ed), 46-54. Animal Welfare Institute, Washington, DC.

Hafez, E.S.E., 1993. Reproducciòn, hormonas y factores de crecimineto. In: Hafez ESE,editor. Reproducciòn e Inseminaciòn Artificial enAnimales. Sixth ed. Mexico, DF: Interameca-McGraw-Hill.

Hansen, L.T., Berthelsen, H., 2000. The effect of environmental enrichment on the behaviour of caged rabbits (Oryctolagus cuniculus). Appl. Anim. Behav. Sci. 68, 163–178.

Hill, E.E., Zack, E., Battaglini, C., Viru, M., Viru, A., Hackney, A.C., 2008. Exercise and circulating cortisol levels: The intensity threshold effect. J. Endocrinol. Invest. 31, 587-591.

Hinde, R.A., 1976. Interactions, relationships and social structure. Man. 11, 1–17.

Holst von, D., 1998. The concept of stress and its relevance for animal behavior. Adv. Study Behav. 27, 1–131.

Hughes, B.O., 1976. Behaviour as an index of welfare. In: Proc. V European Poultry Conference, Malta, pp. 1005-1018.

Ingvartsen, K.L., Andersen, H.R., 1993. Space allowance and type of housing for growing cattle. Acta Agric. Scand. A. Anim. Sci. 43, 65-80.

Jiezierski, T.A., Konecka, A.M., 1996. Handling results in young rabbits. Appl. Anim. Behav. Sci. 24, 157-167.

Jilge, B., Hudson, R., 2001. Diversity and development of circadian rhythms in the European rabbit. Chronobiol. Int. 18, 1–26.

Kersten, A.M.P., Meijsser, F.M., Metz, J.H.M., 1989. Effect of early handling on later open-field behaviour of rabbits. App. Anim. Behav. Sci. 24,157-167.

Koob, G.F., Heinrichs, S.C., 1999. A role for corticotropin releasing factor and urocortin in behavioral responses to stressors. Brain Res. 848, 141-152.

Koolhaas, J.M., Baumans, V., Blom, H.J.M., Von Holst, D., Timmermans, P.J.A., Wiepkema, P.R., 1993. Behaviour, stress and well-being. In: Van Zutphen L.F.M., Baumans V., Beynen A.C., (eds) Principles of Laboratory Animal Science. (1th Edition). Elsevier Science Publisher, Amsterdam, The Netherlands, pp. 75-99.

Kotłowska, M., Kowalski, R., Glogowski, J., Jankowski, J., Ciereszko, A., 2005. Gelatinases and serine proteinase inhibitors of seminal plasma and the reproductive tract of turkey (Meleagrisgallopavo). Theriogenology 63, 1667-1681.

Kovacs, A., Foote, R.H., 1992. Viability and acrosome staining of bull, boar and rabbit spermatozoa. Biotech. Histochem. 67, 119-24.

Kovacs, M., Szendro, Z.S., Milisits, G., Biro-Nemeth, E., Radnai, I., Posa, R., Bonai, A., Kovacs, F., Horn, P., 2006. Effect of nursing method and faeces consumption on the development of bacteroides, lactobacillus and coliform flora in the caecum of the newborn rabbits. Repr. Nutr. Develop. 46, 205-210.

Larsen, C.M., Grattan, D.R., 2012. Prolactin, neurogenesis, and maternal behaviors. Brian Behav. Immun. 26, 201-209.

Lavara, R., Moce, E., Lavara, F., Viudes de Castro, M.P, Vicente, J.S., 2005. Do parameters of seminal quality correlate with the results of on-farm inseminations in rabbits? Theriogenology 64, 1130-1141.

Lavazza, A., Cerioli, M., Grilli, G., 2009. Biosicurezza negli allevamenti cunicoli. Available at: <http://www.asicwrsa.it/documenti/tecnica/Biosicurezza-Coniglio.pdf>

Lawrence, A.B., Rushen, J., 1993. Stereotypic Animal Behaviour: fundamentals and applications to welfare. CAB International, Wallingford Oxon, UK, 212 p.

Lazzaroni, C., Biagini, D., Lussiana, C., 2009. Different rearing systems for fattening rabbits: Performance and carcass characteristics. Meat Sci. 82 (2), 200-204.

Lebas, F., 1968. Mesure quantitative de la production laitière chez la lapine Ann. Zootech. 17, 169-182.

Lehman, M., 1991. Social behaviour in young domestic rabbit under semi-natural conditions. Appl. Anim. Behav. Sci. 32, 269-292.

Lukac, N., Massanyi, P., Krokova, J., Nad, P., Slamecka, J., Ondruska,L., 2009. Relationship between trace element concentrations and spermatozoa quality in rabbit semen. Czech. J. Anim. Sci. 42, 46-50.

Lukac, N., Bardos, L., Stawarz, R., Roychoundhury, S., Makarevich, A.V., Chrenek, P., 2011. In vitro effect on nickel on bovine spermatozoa motility and annexin V-labeled membrane changes. J. Appl. Toxicol. 31, 144-49.

Luzi, F., Xiccato, G., Trocino, A., 2009. Conigli. In: C. Carenzi, M. Panzera (Eds.) Etologia Applicata e Benessere Animale, Vol. 2, Parte Speciale. Le Point Veterinaire Italie, Milano, Italy, pp. 107-120.

Machado, L.C., Martınez-Paredes, E., Paragliola, F., Cervera, C., 2016. Performance of rabbit kits originating from collective and individual cages. In: Proceedings of the 11th World Rabbit Congress. pp. 699–702.

Maertens, L., Lebas, F., Szendro, Zs., 2006. Rabbit milk: a review of quantity, quality and non-dietary affecting factors. World Rabbit Sci. 14, 205-230.

Maranesi, M., Petrucci, L., Leonardi, L., Piro, F., Rebollar, P.G., Millán, P., Cocci, P., Vullo, C., Parillo, F., Moura, A., Mariscal, G.G., Boiti, C., Zerani, M., 2018. New insights on a NGF-mediated pathway to induce ovulation in rabbits (*Oryctolagus cuniculus*). Biol. Reprod. 98 (5), 634-643.

Martin, P.R., Bateson, P.P.G., 1986. Measuring behaviour: an introductory guide. Cambridge University Press.

Mason, G.J., Latham, N.R., 2004. Can't stop, won't stop: is stereotypy a reliable animal welfare indicator? Anim. Welf. 13 (Supplement), S57– S69.

Matics, Zs., Szendro, Zs., Bessei, W., Radnai, I., Biró-Németh, E., Orova, Z., Gyovai, M., 2004. The free choice of rabbits among identically and differently sized cages. In: Proceedings of the 8th World Rabbit Congress, Pueblo, Mexico, 7-10 September 2004 13, pp. 1251-1256.

Mc Bride, A., 2004. Perchè il mio coniglio fa così? Alberto Perdisa Editore, Bologna, Italia, pp.170.
McDonald, D.B., Shizuka, D., 2013. Comparative transitive and temporal orderliness in dominance networks. Behav. Ecol. 24, 511-520.

Meijesser, F.M., Kersten, A.M.P, Wiepkema, P.R., Metz, J.H.M, 1989. An analysis of the open-fields performance of sub-adult rabbits. Appl. Anim. Behav. Sci. 24, 147-155.

Menchetti, L., Brecchia, G., Cardinali, R., Polisca, A., Boiti, C., 2015. Feed restriction during pregnancy: effects on body condition and productive performance of primiparous rabbit does. World Rabbit Sci. 23, 1-8.

Métayer, S., Dacheux, F., Dacheux, J.L., Gatti, J.L., 2002. Comparison, characterization, and identification of proteases and protease inhibitors in epididymal fluids of domestic mammals Matrix metalloproteinases are major fluid gelatinases. Biol. Reprod. 66, 1219-1229.

Monclus, R., Rodel H.G., Palme, R., Von Holst, D., de Miguel, J., 2006. Non-invasive measurement of the physiological stress response of wild rabbits to the odour of a predator. Chemoecology 16, 25-29.

Morisse, J.P., Maurice, R., 1997. Influence of stocking density or group size on behavior of fattening rabbits kept under intensive conditions. Appl. Anim. Behav. Sci. 54, 351-357.

Morisse, J.P., 1998. Le bien-être chez le lapin: rapport de synthèse.In Proc.: 7èmes Journées Recherche Cunicole, Lyon, France, pp. 205-214.

Morrell, J.M., 1995. Artificial insemination in rabbits. Br. Vet. J. 151 (5), 477-488.

Morton, D.B., Jennings, M., Batchelor, G.R., Bell, D., Birke, L., Davies, K., Eveleigh, J.R., Gunn, D., Heath, M., Howard, B., Koder, P., Phillips, J., Poole, T., Sainsbury, A.W., Sales, G.D., Smith, D.J.A., Stauffacher, M., Turner, R.J., 1993. Refinements in rabbit husbandry: second report of the BVAAWF/FRAME/RSPCA/UFAW joint working group on refinement. Lab. Anim. 27, 301–329.

Mousa-Balabel, T.M., Mohamed, R.A., 2011. Effect of different photoperiods and melatonin treatment on rabbit reproductive performance. Vet. Q. 31, 165-171.

Mugnai, C., Dal Bosco, A., Castellni, C., 2009. Effect of different rearing systems and pre-kindling handling on behaviour and performance of rabbit does. Appl. Anim. Behav. Sci. 188, 91-100.

Myers, K., Schneider, E.C., 1964. Observations on reproduction, mortality and behavior in a small, free-living population of wild rabbits. CSIRO Wildl. Res. 9, 138-143.

Mykytowycz, R., 1958. Social behaviour of an experimental colony of wild rabbits *Oryctolagus cuniculus* L.; I establishment of the colony. CSIRO Wildl. Res. 3, 7–25.

Mykytowycz, R., Dudzinski, M.L., 1972. Aggressive and Protective Behaviour of Adult Rabbits Oryctolagus cuniculus (L.) towards Juveniles. Behav. 43, 97-120.

Nagy, Á., Polichronopoulos, T., Gáspárdy, A., Solti, L., Cseh, S., 2015. Correlation between bull fertility and sperm cell velocity parameters generated by computer-assisted semen analysis. Acta Vet. Hung. 63, 370-381.

Naturil-Alfonso, C., Marco-Jiménez, F., Pascual, J.J., Vecente, J.S., 2017. Effect of restricted feeding under rearing on reproduction, body condition and blood metabolites of rabbit does selected for growth rate. World Rabbit Sci. 25, 303-312.

Neumann, C., Duboscq, J., Dubuc, C., Ginting, A., Irwan, A. M., Agil, M., Widdig, A., Engelhardt, A., 2011. Assessing dominance hierarchies: Validation and advantages of progressive evaluation with Elo-rating. Anim. Behav. 82, 911–921.

Noakes, D.E., Parkinson, T.J., England, G.C.W., 2009. Veterinary Reproduction and Obstetrics. Ninth edition. Saunders Elsevier 34, 839-842.

Ombelet, W., Robays, J.V., 2015. Artificial insemination history: hurdles and milestones. Facts Views Vis. Obgyn. 7 (2), 137–143.

Ondruska, L., Parkányi, V., Rafay, J., Chlebec, I., 2008. Effect of LhRH analogue included in seminal dose on kindling rate and prolificacy of rabbits artificially inseminated. In: Proceedings of the 9th World Rabbit Congress, Verona, Italy, p.122.

Orova, Z., Szendrö, Zs., Matics, Zs., Radnai, I., Biró-Németh, E., 2004. Free choice of growing rabbits between deep litter and wire net floor in pens. In: Proceedings of the 8th World Rabbit Congress, Puebla, Mexico, pp. 1263-1265.

Panagiotis, E.S., 2017. Enrichment of Animal Diets with Essential Oils - A Great Perspective on Improving Animal Performance and Quality Characteristics of the Derived Products. Medicines 4 (2), 35.

Pérez-Fuentes, S., Munoz-Silvestre, A., Moreno-Grua, E., Martinez-Paredes, E., Viana, D., Selva, L., Villagra, A., Sanz-Tejero, C., Pascual, J.J., Cervera, C., Corpa, J.M., 2020. Effect of different housing systems (single and group penning) on the health and welfare of commercial female rabbits. Animal 14, 1270-1277.

Poderscek, A.L., Blackshaw, J.K., Beattie, A.W., 1991. The behaviour of group penned and individually caged laboratory rabbits. Appl. Anim. Behav. Sci. 28, 353–363.

Postollec, G., Boilletot, E., Maurice, R., Michel, V., 2006. The effect of housing system on the behavior and growth parameters of fattening rabbits. Anim. Welf. 15, 105-111.

Princz, Z., Radnai, I., Biro-Nemeth, E., Matics, Z., Gerencser, Z., Nagy, I., Szendro, Z., 2008. Effect of cage height on the welfare of growing rabbits. Appl. Anim. Behav. Sci. 114, 284-295.

Quintela, L.A., Pena, A.I., Vega, M.D., Gullón, J., Prieto, C., Barrio, M., Becerra, J.J., Herradón, P.G., 2004. Ovulation induction in rabbit does submitted to artificial insemination by adding buserelin to the seminal dose. Reprod. Nutr. Dev. 44, 79-88.

Quintela, L.A., Peña, A.I., Vega, M.D., Gullón, J., Prieto, C., Barrio, M., Becerra, J.J., Herradón, P.G., 2008. Ovulation induction in rabbit does by intra vaginal administration of the Gnrh analogue [des-Gly10, d-ala6] Lhrh ethylamide: field trial. In: Proceedings of the 9th World rabbit Congress, Verona, Italy, p. 123.

Rebollar, P.G., Alvariño, J.M.R., Illera, J.C., Silván, G., 1997. Effect of gonadoreline and naloxone on induction of ovulation and plasma LH in rabbit. J. Physiol. Biochem. 53, 205-210.

Rommers, J.M., Boiti, C., Jong, I.D., Brecchia, G., 2006. Performance and behaviour of rabbit does in a group-housing system with natural mating or artificial insemination. Reprod. Nutr. Dev. 46 (6), 677–687.

Rommers, J., Gunnink, H., de Jong, I., 2013. Effect of different types of hiding places on aggression among rabbits does in a group-housing system: a pilot study. In: Proceedings of the 18th Internationale Tagung über Haltung und Krankheiten der Kaninchen, Pelztiere und Heimtiere, Celle, Germany.

Rommers, J.M., Reuvekamp, B.J.F., Gunnink, H., de Jong, I.C., 2014. Effect of hiding places, straw and territory on aggression in group-housed rabbit does. Appl. Anim. Behav. Sci. 157, 117–126.

Ruchti, S., Meier, A.R., Würbel, H., Kratzer, G., Gebhardt-Henrich, S.G., , 2018. Pododermatitis in group housed rabbit does in Switzerland - Prevalence, severity and risk factors. Prev. Vet. Med. 158, 114-121.

Rushen, J., Mason, G., 2006. A Decade or More's Progress in Understanding Stereotypic Behaviour. In: Mason, G., Rushen, J. (Eds.). Stereotypic animal behaviour: fundamentals and applications to welfare, CABI.

Saacke, R.G., White, L.M., 1972. Semen quality and their relationship to fertility. In: Proceedings of the 4th NAAB technical conference on animal reproduction AI, 1972, p. 23-27.

Sainsbury, D., 1986. Farm Animal Welfare. Cattle, pigs and Poultry. Collins, London, UK.

Sajjad, M., Ali, S., Ullah, N., Anwar, M., Akhter, S., Andrabi, S.M.H., 2007. Blood serum testosterone level and its relationship with scrotal circumference and semen characteristics in Nilli-Ravi buffalo bulls. Pak. Vet. J. 27(2), 63-66.

Schepers, F., Koene, P., Beerda, B., 2009. Welfare assessment in pet rabbits. Anim. Welf. 18, 477-485.

Schjelderup-Ebbe, T., 1922. Beiträge zur Sozialpsychologie des Haushuhns. Z. Psychol. 88, 226–252.

Selzer, D., Lange, K., Hoy, S., 2001. Untersuchungen zur Mutter-Kind-Beziehung bei Hauskaninchen unter Berücksichtigung verschiedener Haltungsbedingungen. In: Proceedings of the 12th Symposium on Housing and Diseases of Rabbits, Furbearing Animals and Pet Animals Celle, 106–114.

Sevi, A., 2009. Animal-based measures for welfare assessment. Ital. J. Anim. Sci. 8, 904-911.

Smelser, G.K., Walton, A., Whetam, E.O., 1934. The effect of light on ovarian activity in the rabbit. Journ. Physiol. 80, 174.

Stull, C.L., McDonough, S.P., 1994. Multidisciplinary approach to evaluating welfare of veal calves in commercial facilities. J. Anim. Sci. 72, 2518-2524.

Szendro, Zs., 2009. The relationship between housing systems and animal welfare. In: Proc. Giornate di Coniglicoltura ASIC 2009, pp. 25-39. Available on: http://www.asic.wrsa.it/documenti/giornate2009/03_Szendro.pdf.

Szendrő, Z., McNitt, J.I., 2012. Housing of rabbit does: Group and individual systems: A review. Livest. Sci. 150 (1-3), 1–10.

Szendro, Z., Miko, A., Odermatt, M., Gerencser, Z., Radnai, I., Dezsery, B., Garai, E., Nagy, I., Ndrondro, K., Matics, Z., 2013. Comparison of performance and welfare of single-caged and group-housed rabbit does. Animal 7, 463–468.

Szendrő, Zs., Gerencsér, Z.S., McNitt, J.I., Matics, Z.S., 2016. Effect of lighting on rabbits and its role in rabbit production: A review. Livest. Sci. 183, 12-18.

Theau-Clément, M., 2007. Preparation of the rabbit doe to insemination: a review. World Rabbit Sci. 15 (2), 61 – 80.

Theau-Clément, M., 1998. La fecondazione artificiale della coniglia. Large Animals Review, 4 (4).

Theau-Clément, M., 2007. Preparation of the rabbit doe to insemination: a review. World Rabbit Sci. 15 (2), 61–80.

Theau-Clément, M., Lebas, F., Beckers, J.F., Drion, P.V., 2008. Evolution of anti-eCG antibodies in response to eCG doses and number of injections. Relationship with productivity of rabbit does. Animal 2, 746-751.

Theau-Clément, M., Guardia, S., Davoust, C., Galliot, P., Souchet, C., Bignon, L., Fortun-Lamothe, L., 2016. Performance and sustainability of two alternative rabbit breeding systems. World Rabbit Sci. 24 (4), 253-265.

Trocino, A., Xiccato, G., Queaque, P.I., Sartori, A., 2004. Group housing of growing rabbits: effect of stocking density and cage floor on performance, welfare and meat quality. In: Proceedings of the 8th World Rabbit Congress, Puebla City, Mexico, pp. 1277-1281.

Trocino, A., Xiccato, G., 2006. Animal Welfare in reared rabbits: a review with emphasis on housing system. World Rabbit Sci. 14, 77-93.

Trocino, A., Majolini, D., Tazzoli, M., Filiou, E., Xiccato G., 2013. Housing of growing rabbits in individual, bicellular and collective cages: fear level and behavioural patterns. Animal 7, 633-639.

Trocino, A., Zomeño, C., Filiou, E., Birolo, M., White, P., Xiccato, G., 2019. The use of environmental enrichments affects performance and behavior of growing rabbits housed in collective pens. Animals 9 (8), 537.

Verga, M., Carenzi, C., 1981. Il comportamento degli animali domestici. Edagricole, Bologna, Italia, pp. 159.

Verga, M., Le Neindre, P., Moynagh, J., 1999. Atti Convengo Internazionale: parliamo di benessere e allevamento animale, Cuneo, Italia, pp. 5-16.

Verga, M., Luzi, F., Carenzi, C., 2007. Effects of husbandry and management systems on physiology and behaviour of framed and laboratory rabbits. Horm. Behav. 52, 122-129.

Verga, M., Luzi, F., Petracci, M., Cavani, C., 2009. Welfare aspects in rabbit rearing and transport. Ital. J. Anim. Sci. 8, 191-204.

Verwer, C.M., van Amerongen, G., van den Bos, R., Hendriksen, C.F.M., 2009. Handling effects on body weight and behavior of group-housed male rabbits in a laboratory setting. Appl. Anim. Behav. Sci. 117, 93-102.

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

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

Williamson, C.M., Lee, W., Curley, J.P., 2016. Temporal dynamics of social hierarchy formation and maintenance in male mice. Anim. Behav. 115, 259–272.

Xiccato, G., Trocino, A., 2007. Italia, un sistema de producciòn cunicola integrada. In: Proceedings of the II Congreso Iberico de Cunicultura, Vila-Real, Portugal, pp. 163-172.

Yoshimura, Y., Nakamura Ando, Y.M., Jinno, M., Nanno, T., Oda, T., 1992. Protein kinase C mediates gonadotropin-releasing hormone agonist induced meiotic maturation of follicle-enclosed rabbit oocytes. Biol. Reprod. 47, 118-125.

Zapletal, D., Pavlik, A., 2008. The effect of lecirelin (GnRH) dosage on the reproductive performance of and lactating rabbit does. Anim. Reprod. Sci. 104 (2-4), 306-315.

Zhang, J., Boyle, M.S., Smith, C.A., Moore, H.D.M., 1990. Acrosome reaction of stallion spermatozoa evaluated with monoclonal antibody and zona-free hamster eggs. Mol. Reprod. Dev. 27, 152-158.

Zhang, Y.Q., Qin, Y., 2012. Reproductive performance of rabbit does by adding leuprorelin in semen to induce ovulation. In: Proceedings of the 10th World Rabbit Congress, pp. 419-423.

Zomeño, C., Birolo, M., Gratta, F., Zuffellato, A., Xiccato, G., Trocino, A., 2018. Effects of group housing system, pen floor type and lactation management on performance and behaviour in rabbit does. Appl. Anim. Behav. Sci. 203, 55-63.

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(Marcel Proust)

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