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Management Factors Affecting Fertility in Sheep

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1. Introduction

An Arab horse breeder in the early 13th century carried out the first insemination reported, by trapping stallion semen in wool placed in the vagina of a mare and transferring this to the vagina of another mare (Heape, 1898). Later, in 1780, an Italian priest and physiologist named Lazzaro Spallanzani performed artificial insemination with dog semen, and revolutionised the way scientists thought. Since then, scientist and farmers have striven to improve this technology, motivated by the benefits that could be achieved. Sheep is one of the species subsequently linked to this technology and in which many questions still remain to be resolved to improve fertility. However, the potential impact of this technique on the genetic progress of sheep is high and further studies are needed to improve its efficiency.

In Spain, artificial insemination programmes in sheep are linked to the genetic selection schemes of the breeds, but it has not been successfully integrated with reproductive technology on farms as happen in sows or cows. The technical difficulty and weak fertility, ranged between 15 to 60 % for pregnancy rate, limits its application. In Spain, most commercial programmes use refrigerated semen (15 degrees C) by superficial intracervical deposition (cervical), whereas the use of frozen-thawed semen by intrauterine deposition (laparoscopy) is more restricted. Cervical insemination with fresh semen is the main method chosen due to its simplicity and satisfactory results. With the aim of improving its efficiency, this paper focuses on identifying the main management effects affecting AI results when this technology is applied.

2. Female associated factors

Management factors associated with artificial insemination in the ewe can modify fertility. In reproductive planning, intervals between lambings, season, age of ewe, heat stress, nutrition state or breed are some of the factors which have a great effect on fertility results. David et al. (2008), using a joint model combining two main traits, one relative to female and the other relative to the male, reported that the main variation factors of AI success were relative to non-sex-specific effects and to female effect, suggesting that choosing females to inseminate might slightly improve the AI results.

2.1 Season

Seasonal variations are described as a limiting factor in sheep reproduction. In natural conditions, seasonality, which is mediated by photoperiod, modifies hormonal balance and causes seasonal reproductive variations in sheep (Karsch, et al. 1984; Yeates, 1949), giving rise to a decrease in reproductive activity during long days (anoestrous season). Photoperiodic information is translated into neuroendocrine changes through variations in melatonin secretion from the pineal gland (Bittman, et al., 1983). Melatonin, secreted in pineal gland, triggers variations in the secretion of luteinising hormone-releasing hormone (GnRH), luteinising hormone (LH) and follicle stimulating hormone (FSH) (Arendt, et al., 1983, Karsch, et al., 1984). In any case, seasonal changes in reproductive activity are clearly defined in sheep breeds from high latitudes (>40º)(Pelletier, et al., 1987), where the differences in daylight duration between short days and long days are more notable.

As in natural mating, season affects fertility after AI, although hormonal treatment is used to synchronise and induce oestrus. Windsor (1995) reported low cervical AI fertility rates in non-breeding season in Merino ewes, a shallow seasonal breed. According to this, Anel et al. (2005) found a season effect on the AI fertility in Churra ewes, which was more important in cervical than laparoscopic artificial insemination. In cervical AI, semen is deposited in the external portion of the cervix and the sperm transport is affected by cervical mucus quality. Theses authors suggest that photoperiod could alter progestagens and so cervical mucus characteristics, making it scarcer and more viscous. In consequence, sperm transport in the cervix can be interfered with. It is important to note that seasonality affects the ram reproductive parameters in the same way and changes in seminal quality during anoestrous season may decrease the fertility results after AI.

Moreover, subcutaneous melatonin implants are widely used to bring the breeding season forward and improve reproductive performance in non-breeding season (Chemineau, et al., 1991; Haresign, et al., 1990). Melatonin treatments act by mimicking a short-day-like response (O'Callaghan, et al., 1991) and induce oestrus during the non-breeding season. Not only an increase in the percentage of pregnant ewes (fertility) has been described after melatonin implant treatment in anoestrous season, but also the number of lambs born per ewe (litter size) (Abecia, et al., 2007; Arrebola, et al., 2009; Chemineau, et al., 1992). This improvement could be due to a higher rate of embryonic survival, an improvement in luteal function or a reduction in the antiluteolytic mechanisms (Abecia, et al., 2008). In AI, a fertility rate increase has been reported with melatonin implant treatments (Laliotis, et al., 1998; Legaz, et al., 2000) after cervical AI.

2.2 Age

Another important factor affecting fertility after cervical AI is ewe age. In comparison with adult ewes, young and maiden ewes have lower fertility, probably due to impaired sperm transport combined with low mucus production in the cervical canals during oestrus (Selaive-Villarroel & Kennedy, 1983a, b). After that, most studies have described a decrease in AI success with increasing female age. Shackell et al. (1990), predicted 2-3% fertility reduction per year of age for different breeds. Esmailizadeh et al., (2009) reported that as the age of the ewes increased from 2 to 7 years, the proportion of barren ewes significantly decreased from 29 to 5%. In a more recent study in the Spanish Churra dairy breed, Anel et al. (2005), described how, after 1.5 years of age, the lambing rate decreased by 1.74% per year for cervical AI. The highest rates of fertility declining with age were described in the Lacaune breed by Colas et al. (1973), who reported a drop in fertility of 15% per year.

Paulenz et al. (2007), observed that the age of the ewes had a significant effect on the non-return rate, but not on lambing rate, whereas in Fukui et al. (2010), both the pregnancy and lambing rates in the ewes significantly declined as age increased. The detrimental effect of increased fertility age could be explained by the fact that aged ewes have increased risks of reproductive disorders and decreased ovulation rates with quality ovulated oocytes compared with younger ewes.

The question is: what is the optimal age for cervical AI? Alabart et al. (2002) , studied the influence of age on fertility in a total of 3819 Rasa Aragonesa ewes aged 1 to 12 years. In this study, maximum fertility (56.7%) was observed at 3 years, and ewes aged from 2 to 5 years (79.5% of the inseminated ewes) had mean fertility values above 50%. These results partially confirm the observations by Colas et al. (1973), who reported a significant decrease in the fertility of ewes inseminated when over 3.5 years old, and Gabiña and Folch (1987), who observed a strong fall in the fertility of ewes inseminated at 4 or more years of age. Anel et al. (2005) recorded the best fertility rates in ewes aged between 1.5 and 4.5 years; beyond this age, fertility declined remarkably. Fertility also decreased depending on the number of previous parturitions. In other studies, the maximum fertility was obtained at around 2 years of age, with a progressive fall afterwards (Fantova, et al., 1998). It may be concluded that insemination groups should be made up of 2 to 5 year old ewes while younger and older ewes should be used for natural mating.

2.3 Lambing-AI interval

The need for a resting period for the ewe after lambing to allow uterine involution is well known. However, sometimes the increasing reproductive rate imposed by the demanding production system involves short resting periods from lambing to AI, which affects fertility in a negative way. According to Bodin et al. (1999), reducing the lambing-AI interval to below 40-50 days induces a significant decrease in fertility, even after natural mating. Most authors recommend not inseminating ewes any sooner than 50 days post-partum (Anel, et al., 2005).

2.4 Breed

Ewe breed is also a significant source of variation in fertility after AI (Donovan, et al., 2004; Fukui, et al., 2007; Papadopoulos, et al., 2005; Salamon & Maxwell, 1995). Differences in the mean time of ovulation and ovulation rates in different breeds of ewes at differences locations may explain the variation in fertility (Salamon & Maxwell, 1995).

Alternatively, variation may be due to differences in the morphometric characteristics of the cervix (Eppleston, et al., 1994). In this sense, Kaabi et al. (2006), carried out a morphometric study in four ovine breeds (Assaf, Churra, Castellana and Merino) showing important differences in length, width, number of folds and distance between folds, which originates breed variations in the depth of catheter penetration into the cervix during AI. In this study, the breeds yielding lower fertility after AI resulted in higher cervical complexity, and achieved a lesser degree of cervical penetration of the catheter during cervical AI. As explained below, different studies have found a positive correlation between cervical AI depth and fertility (Kaabi, et al., 2006).

2.5 Body weight and body condition score

For an adequate response in a breeding programme, ewes must be suitably nourished and maintained in good body condition. Clearly, ewes with a good nutrient intake respond most

rapidly to the onset of the breeding season and continue to respond with an increase in ovulation rate (Keisler & Buckrell, 1997). Flushing is understood as the rapid increase in ovulation rate of ewes receiving a nutrient supplementation before mating. Under harsher nutritional conditions in the semi-arid southern Mediterranean region, where regular food supply is not guaranteed, lambing and twinning rates were shown to be boosted following nutritional flushing (Younis, et al., 1978; Landau & Molle, 1997; Branca, et al., 2000) or when the live weights of the ewes were higher at mating (Gunn & Doney, 1979, Thomson & Bahhady, 1988).

However, Lassoued et al. (2004), showed important interactions between genotype and level of nutrition. In this sense, in highly prolific ewes like D'Man breed, higher levels of nutrition prior to and during mating were associated with improved reproductive performance, but in low prolific breeds such as Queue Fine de l'Ouest, neither ovulation rate nor lambing rate were affected by the dietary treatment. In a recent work by Fukui et al. (2010) body weight did not significantly affect fertility.

Body condition score (BCS) has proved useful as a management tool for subjectively assessing the nutritional status of ewes. In this way, body condition directly affects hypothalamic activity and GnRH secretion, but not pituitary sensitivity to GnRH, and these effects on reproductive performance are also mediated through changes in ovarian hormones or in hypothalamo-pituitary sensitivity to ovarian hormones (Rhind, et al., 1989). The effect of body condition on the ovulation rate of ewes has been extensively reported (Ducker & Boyd, 1977; Morley, et al., 1978; Adalsteinsson, 1979; Gunn & Doney, 1979; Gonzalez, et al., 1997). High body condition score has been associated with an increase of ovulation (Rhind, et al., 1989; Xu, et al., 1989), especially in Mediterranean breeds at the beginning of the breeding season (Forcada, et al., 1992). Most authors recommend a BCS of 2.5 to 3.0 either for natural or artificially breeding (Contreras-Solis, et al., 2009; Husein & Ababneh, 2008). In a study carried out in inseminated Rasa Aragonesa ewes (Bru, et al., 1995), the lowest pregnancy rates (32,7 %) were obtained in sheep with a BCS<2, the average values (48.3%) with BCS between 3 and 2 and the higher values (58.8%) when BCS was >3. The importance of BCS in fertility has been also reported in Spanish Manchega breed (Montoro, 1995).

Fukui et al. (2010), , concluded that body nutritional condition is an important factor, next to ewe age, influencing the fertility of ewes after AI regardless of body weight. Nulliparous ewes less than 3 years old and with a BCS of more than 3.0 are expected to have higher fertility than other types of ewes.

2.6 Farm/Herd

Different ewe farms have different management practices and this may have an impact on fertility after AI. Reproductive planning (intervals between lambings, season, age of first mating, AI technique, etc.) and animal handling (feeding, health, preparation of AI lots, etc.) have a great effect on fertility results (Anel, et al., 2005). The significant effect of the farm has been described in several studies (Anel, et al., 2005, Fantova, et al., 1999, Paulenz, et al., 2002).Thus, in order to improve fertility results, handling conditions on farms must be improved, along with more widespread use of AI techniques.

The geographical area where the farm is located may also have an influence on the success of AI. In a recent study carried out in north-eastern Spain (Palacin et al., 2011), data from 18.528 AI in Rasa Aragonesa ewes belonging to a selection scheme with similar management

were recorded in order to analyse the effect of farm geographical location and the theoretical time distance between the farm and insemination centre on the fertility after cervical AI. An average fertility of 54.3% was observed, with significant differences among the 14 regions studied. Fertility rates higher than 60% were found in the northern regions near the Pyrenees Mountains and the lowest results (38.5-48.3%) were obtained in the southern regions. The average time distance of these regions did not differ. The regions nearest to the insemination centre, with similar climatic conditions, showed medium fertility rate (54.0-57.8%). These results showed a huge variability after insemination, taking into account the geographical location of the farm.

2.7 Heat stress

It has been reported than in tropical and sub-tropical areas the local sheep show restricted sexual activity in the summer months (Marai et al., 2004). Marai et al., 2007 reviewed how exposure to high ambient temperature causes impairment of reproductive functions in sheep. The heat effect is aggravated when heat stress is accompanied with high ambient humidity (Marai et al., 2000, 2004, 2006, 2007). Heat stress evokes a series of drastic changes in animal biological functions, which include a decrease in feed intake efficiency and use, disturbances in the metabolism of water, protein, energy and mineral balances, enzymatic reactions, hormonal secretions and blood metabolites. (Shelton, 2000; Marai et al., 2006).

2.8 Synchronization treatment

Oestrous synchronisation and ovulation induction treatments are widely spread in AI in order to control the optimum insemination time. Oestrous behaviour in small ruminants is not clearly shown, so treatments are needed to prevent an asynchrony between ovulation and insemination time, which may be the commonest cause of failure of artificial insemination programmes (Jabbour & Evans, 1991).

Synchronisation treatments are a useful tool not only for AI programmes but also in natural mating, particularly to ensure lambing in anoestrous season. However, many studies report that synchronisation causes reduced fertility after cervical AI (Robinson, et al., 1970; Hawk, 1971) and after natural mating (Quinliva.Td & Robinson, 1969; Hawk & Conley, 1975; Allison & Kelly, 1978). Different hormonal treatments have been used in the control of sheep reproduction, but progestagen analogues (fluorogestone acetate and medroxyprogesterone acetate) are the most commonly used to induce and synchronise oestrus in natural mating or AI of small ruminants (Lunstra & Christenson, 1981; Pearce & Robinson, 1985; Langford, et al., 1982; Baril, et al., 1993, Greyling, et al., 1997). Progestagens produce a mimetic effect of the luteal phase of the oestrous cycle and a sudden progestagen removal followed by administration of an equine chorionic gonadotropin (eCG) dose (FSH- and LH-like stimulation) induces oestrous activity. Currently, intravaginal progestagen-impregnated devices (sponges or CIDR) for 12-14days followed by administration of 250-500IU eCG has been proposed (Abecia, et al., 2011) as a synchronised treatment in sheep, in which insemination can be performed from 47h (intrauterine) to 55 (cervical) hours after device removal.

Although long-term progestagen treatment results in efficient oestrous synchronisation, high variability has been reported in fertility (Vinoles, et al., 2001; Menchaca & Rubianes, 2004;). Progestagen treatment appears to result in an asynchrony between oestrus and ovulation (Scaramuzzi, et al., 1988; Sirois & Fortune, 1990) and reduce sperm transport

through the cervix (Killen & Caffery, 1982; Armstrong & Evans, 1984; Pearce & Robinson, 1985). Other studies have reported lower fertility rate caused by the negative effect of long-term progesterone treatment on oocyte development (Vinoles, et al., 2001; Menchaca & Rubianes, 2004) related to subluteal progesterone levels.

According to this, recent research efforts are focused on shortening synchronisation treatments. Shortening treatment (5-6days) with different progestagen devices seems to be enough to achieve efficient oestrous synchronisation in natural mounting both during the anoestrous season (Ungerfeld & Rubianes, 1999) and in breeding season (Vinoles, et al., 1999; Ustuner, et al., 2007) with a similar fertility rate to long-term treatment. Nevertheless, the interval between progestagen device withdrawal and the onset of oestrus was shortened (Ungerfeld & Rubianes, 1999; Vinoles, et al., 2001; Zeleke, et al., 2005; Ustuner, et al., 2007) due to a delay in corpus luteus regression in cyclic ewes (Menchaca & Rubianes, 2004). This asynchrony could decrease the success of artificial insemination programmes, so a treatment that ensures an acceptable luteolysis seems to be necessary to enhance the oestrous synchronisation (Ustuner, et al., 2007).

The luteolytic effect of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) (McCracke, et al., 1972) and its analogues has been used to control corpus luteus activity. In goats, the use of a short-term progestagen protocol combined with $PGF_{2\alpha}$ administered at progestagen sponge insertion time was successful in artificial insemination with frozen-thawed semen (Corteel, et al., 1988). In sheep, Beck et al (1993) reported acceptable oestrous synchronisation and fertility results in natural mating using $PGF_{2\alpha}$ treatment combined with short-term progestagen. Nowadays, $PGF_{2\alpha}$ treatment use in small ruminant reproduction control has increased because of narrow restrictions on the use of progestagens in animal production both in the United States and the European Union (Martin, et al., 2004; Menchaca & Rubianes, 2004). $PGF_{2\alpha}$ is quickly metabolised with a minimum residue level (Light, et al., 1994). In some South American countries, short progestagen treatment with $PGF_{2\alpha}$ administration is used in goat management (Menchaca & Rubianes, 2004). However, the effectiveness of $PGF_{2\alpha}$ or its analogues depends on the ovary status (Houghton, et al., 1995) and is not very useful during the anoestrous season (Acritopoulou & Haresign, 1980). Moreover, a varied response and low pregnancy rates in AI have been reported with single $PGF_{2\alpha}$ treatment or progestagen combined (Boland, et al., 1978; Hackett, et al., 1981; Olivera-Muzante, et al., 2011), so with the current methods it is not an appropriate synchronisation treatment for AI unless previous oestrous detection is carried out.

Another factor affecting fertility after cervical AI related with hormonal synchronisation is the development of anti-eCG antibodies. Females involved in repeated treatment throughout their reproductive life, particularly those involved in AI genetic programmes or intensive lambing systems, develop anti-eCG antibodies (Baril, et al., 1996; Bodin, et al., 1997; Roy, et al., 1999). These antibodies are associated with low reproductive rates, especially in fixed time AI in sheep (Maurel, et al., 2003) and goats (Baril, et al., 1996), and in multiple ovulated ewes (Forcada, et al., 2011). This is less pronounced when repeated treatment is combined with natural mating. High concentrations of anti-eCG antibodies are reported with a lack or delay in oestrus and pre-ovulatory LH surge (Baril, et al., 1993; Roy, et al., 1999; Maurel, et al., 2003), decreasing the fertility after AI.

Alternatives for reproductive activity control that ensure optimum oestrous synchronisation and successful AI results are needed in sheep, in line with current demands in public and animal welfare.

3. Male associated factors

The ram may greatly influence fertility results after cervical AI. It has been reported that variation in fertility of ram ejaculates exists independently of the sperm quality (Choudhry, et al., 1995; Paulenz, et al., 2002). Variations in the fertility of rams have been reported after cervical inseminations with fresh semen (Anel, et al., 2005; Paulenz, et al., 2002), with frozen semen (Colas, 1979; Windsor, 1997; Soderquist, et al., 1999; Paulenz, et al., 2005, 2007) and after laparoscopic inseminations with frozen semen (Eppleston et al., 1986; Maxwell, 1986; Eppleston, et al., 1991; Eppleston & Maxwell, 1995). In a large scale epidemiological study, Anel et al. (2005) observed that the male factor significantly influenced fertility. Despite the restrictions in the choice of ejaculates, the authors found important differences in fertility among rams, particularly when cervical AI with cooled semen was used. Salamon and Maxwell (1995) proposed that ram differences in fertility could be both genetic and environmental, whereas ejaculate differences are probably due to nutrition, management and previous frequency of ejaculation.

Whereas differences in fertility have been demonstrated among fertile males in different species, the causes of these differences remain unclear (Ostermeier, et al., 2001). Saacke et al. (1988, 1994) have suggested in bulls that factors associated with semen quality which affect fertility can be classified as either compensable or non-compensable. It was suggested that the effects of compensable factors on fertility might be sensitive to the number of sperm inseminated, whereas those of non-compensable factors were not. As the number of sperm inseminated increases, fertility increases until a plateau is reached (den Daas, 1992). At this point, compensable factors no longer have an effect on fertility. Commercial insemination of ovine in Mediterranean Countries provides at least the plateau number of sperm in an insemination dose. It is thus the non-compensable factors that contribute most to the fertility level of a ram. A non-compensable defect in sperm would be one in which a sperm reaches the fertilisation site and initiates the egg activation process, but fails to sustain zygotic, embryonic, or foetal development (Ostermeier, et al., 2001). Evidence of such defects in sperm has been described in bulls with fertility differences (Eid, et al., 1994). Likely candidates for non-compensable factors would be incorrectly assembled chromatin or damaged DNA within the sperm nucleus. It seems logical to assume that the transfer of a complete and intact DNA molecule from sperm to ovum is crucial to obtain fertilisation with certain prospects of success. It is well-known that the presence of defects in the genetic material, such as anomalies in chromatin condensation related with the sperm maturation process, the integrity of the DNA molecule associated with the presence of breaks both of single and double DNA strands, or the presence of chromosomal anomalies, are closely associated with infertility (Aravindan, et al., 1997).

Season

Although seasonality is less marked in male than in female, changes in testicular volume, hormonal profiles, sexual behaviour and semen quality that affect the reproductive performance of rams have been reported (Casao, et al., 2010a). In this sense, the treatment of rams with slow release implants of melatonin during the non-breeding season accounted for increased scrotal diameter and improved the reproductive performance of ewes inseminated during anoestrus with semen from these melatonin-implanted males. A direct beneficial action of melatonin on sperm motility (Casao, et al., 2010c) and on other ram sperm characteristics during the non-breeding season has recently been demonstrated, with decreased apoptotic-like changes and modulating capacitation and fertilisation rates (Casao, et al., 2010b).

Nutrition

Several studies on nutrition in rams have demonstrated that diet may have an effect on testis size and sperm production (Brown, 1994). It also has been described that specific components of the diet, such as Vitamin E, may have a positive effect in increasing semen quality and quantity (Yue, et al., 2010). The effect of diet of the rams on the reproductive success of ewes after AI remains to be determined.

4. Artificial insemination-associated techniques

Inadequate semen preservation and difficulty in passing through the cervix during AI are the major obstacles to the extensive use of cooled or cryopreserved semen in sheep AI programmes (Yaniz, et al., 2010, 2011). Exo-cervical deposition of diluted liquid ram semen, preserved at 15∘ C for less than 8 h, is currently the AI technique predominantly used in the Mediterranean countries (Lopez-Saez, et al., 2000; Yaniz, et al., 2010, 2011).

4.1 Semen collection
4.1.1 Semen collection frequency

Semen collection frequency may have an impact on sperm quality. Long abstinence periods (Pascual, 1993) and successive ejaculations (Ollero et al., 1994) have been associated with membrane alterations of spermatozoa. A decrease in semen volume and sperm concentration with successive ejaculations has been reported in several studies (Ollero et al., 1996; Kaya et al., 2002). In the study by Ollero et al. (1996), the maximum proportion of viable cells was obtained in the second ejaculate after an abstinence period of 3 days. The authors concluded that the use of the second and/or a mixture of second and third ejaculates would improve the results in artificial insemination. The general recommendation is to establish a routine of semen collection, for example of two-three times per week (two collections per day/per ram) on different and non-consecutive days, independently of the use of the semen obtained. Increased semen collection frequency may have an effect on sperm quality and the composition of the seminal plasma (Kaya et al., 2002), although it remains to be determined whether this has an impact on field fertility. In this sense, the procedure of taking one or two collections per day from each ram during the working week (Monday-Friday), with a 2-day rest period during the weekend, has been described in Ireland (Gordon, 1997).

4.1.2 Hygienic conditions

Semen collection in farm animal species is not a sterile procedure, and some degree of contamination with bacteria cannot be avoided (Clément et al., 1995; Varner et al., 1998; Althouse et al., 2000; Thibier and Guerin, 2000; Althouse and Lu, 2005; Aurich and Spergser, 2007; Bielanski, 2007; Yániz et al., 2010). In rams, semen is usually collected with an open-ended artificial vagina, which may be contaminated with bacteria from the surface of the penis and prepuce, collection area, equipment and people. As a consequence, bacteria might compromise semen quality during storage and contaminate the female's reproductive tract. We have recently described that ram semen is normally colonised by a variety of microorganisms that may reduce semen preservation and fertility (Yániz et al., 2010). In particular, the contamination of ram semen with enterobacterial species reduced sperm quality during storage at 15 ∘C in a concentration-dependent manner.

Different strategies may be taken to minimise the effects of bacterial contamination on extended semen, as the bacterial concentration remains below a threshold level, so fertility is not affected (Althouse et al., 2000). The first and most viable option is to enhance the hygienic measures during semen collection and processing. Dilution of the ejaculates with sterile diluents will further decrease the concentration of contaminants (Bielanski, 2007), although this aspect has low influence in ovine because of the high sperm concentration employed for AI. Finally, control of bacterial growth is usually performed by the use of semen extenders containing antibiotics with broad-spectrum bactericidal or bacteriostatic activity (Maxwell and Salamon, 1993; Salamon and Maxwell, 2000). Perhaps too much reliance is often placed on this method of bacterial control in ovine semen. In this species, necessarily short storage periods for semen determine that the control of bacterial multiplication may be less important than in other animal species in which successful long-life semen extenders have been developed. Interestingly, in a recent study (Yániz et al., 2010), it was observed that 13% of identified bacteria were simultaneously resistant to penicillin and streptomycin, the most common preservative antibiotic combination used in ovine semen extenders, whereas *E. coli* , the bacteria with the highest impact on sperm quality, was frequently resistant to both antibiotics (31.7 %, 13/41). Antibiotics with higher antimicrobial activities were gentamycin and ceftiofur, and their inclusion in ram semen diluents should be considered.

4.2 Semen evaluation

Semen evaluation is a useful tool in the selection of males and ejaculates for assisted reproduction. Traditional evaluation techniques, based on the subjective assessment of parameters such as sperm motility and morphology, semen volume or concentration, have long been employed in the diagnosis of male subfertility and sterility (Verstegen et al., 2002). An *in vitro* system that could accurately predict field fertility would facilitate stricter selection of AI rams with regard to the semen quality and would provide a valuable tool for increasing conception rate (Donovan et al., 2004). However, finding a laboratory test reliable enough to predict the potential fertility of a given semen sample or a given sire for AI is still considered utopian, as indicated by the modest correlations seen between results obtained in vitro and field fertility (Rodríguez-Martínez, 2003). Male fertility is complex, and depends upon a heterogeneous population of spermatozoa interacting at various levels of the female genital tract, the vestments of the oocyte, and the oocyte itself (Rodríguez-Martínez, 2003). For this reason, laboratory assessment of semen must include the testing of as many relevant sperm attributes for fertilisation and embryo development as possible, not only in individual spermatozoa but also within a large sperm population (Rodríguez-Martínez, 2003). In practice, routine sperm analysis requires fast, objective and accessible methods of assessing different aspects of sperm viability (Yániz et al., 2008). In this sense, the common use of computer-assisted sperm analysis (CASA) methods for sperm motility, of image analysis for the evaluation of membrane integrity (Yániz et al., 2008), of DNA fragmentation with sperm chromatin diffusion techniques (SCD), (Gosalvez et al., 2008; López-Fernández et al., 2008), or fluorimetry, etc., would theoretically improve the predictive capacity of semen analysis, although more studies are needed to determine the utility of these techniques in the practical use of AI.

4.3 Sperm number per AI dose

The difficulty in passing through the cervix during AI due to the type of cervical canal found in this species determines that semen can only be deposited inside the cervix, usually

in the external portion. The retention capacity of the ovine cervix is very low (0,1-0,2 ml) (Gordon , 1997), whereas a large sperm number per dose is required to compensate the huge barrier effect of the cervix (around 400 x 10^6 sperm/dose in Spanish AI).

It is important to determine the minimal sperm dose per insemination in order to maximise the genetic diffusion of males, without decreasing AI success. In the study by Langford and Marcus (1982), fertility after insemination of 400 or 200 x 10^6 spermatozoa was similar to that observed after natural service at progestagen-induced oestrus. However, when less than or equal to 100 x 10^6 spermatozoa were inseminated, fertility fell markedly and the number of lambs per ewe inseminated decreased. These data indicate that insemination of 200 x 10^6 spermatozoa, i.e. less than 10% of the number in a single ram ejaculate, allows normal conception rates in progestagen-treated ewes. It seems that the minimal sperm dose may be between more than 100 and 200 x 10^6 (120 million sperm in Australian works, Gordon, 1997), although a breed-effect should not be discarded, as differences in the cervix anatomy have been described.

4.4 Semen preservation
4.4.1 Diluents
Despite numerous past efforts to improve semen diluents, few new additives have been introduced in the extender composition for ram semen (Yániz et al., 2005). Biological components such as milk or egg yolk in the diluent have not really been effective in the applied use for AI. So, for example, skimmed milk, a complex and variable biological component, is still the main diluent used to preserve sheep semen at 15 °C for AI in numerous countries (López-Sáez et al., 2000; Yániz et al., 2011). The basic components of semi-synthetic diluents for the liquid storage of ram semen (buffers combined with sugars and egg yolk), have changed little since those first used in the early 20th century (Maxwell and Salamon, 1993).

The predominant empirical approach of most of the studies could partially explain the slow advances made in the development of chemically defined extenders for ram semen storage. An individual study on the effect of each extender component on the viability of the sperm cell could greatly contribute to the development of a more rational synthetic diluent. For example, in a recent work (Yániz et al., 2011) we studied the effect of different buffer systems included in the composition of well-defined and synthetic diluents (in vitro), on sperm quality parameters during storage at 15°C. TRIS caused drastic modifications of certain sperm kinematic parameters during storage at 15°C although, along with citrate, it is one of the main buffers included in the composition of semi-synthetic ram semen diluents (López et al.,1999; López-Sáez et al., 2000; Salamon and Maxwell, 2000; Paulenz et al., 2003; Martí et al., 2003; Yániz et al., 2008).

With the available diluents, semen preservation in the non-frozen state is limited to 6-8h without reducing fertility (Maxwell and Salamon (1993); Salamon and Maxwell, 2000). Irrespective of the diluent, dilution rate, storage temperature or conditions, the sperm deteriorated as the storage duration increased. The main changes that occurred during storage included reduction in motility and morphological integrity of sperm. These changes may be attributed to the accumulation of the toxic products of metabolism, mainly of reactive oxygen species (ROS) that cause lipid peroxidation of the sperm plasma membranes. The above events are accompanied by a decline in transport and survival of spermatozoa in the female reproductive tract and reduction in fertility (Salamon and

Maxwell, 2000). When longer period of storage are required, the use of reduced temperature (about 4ºC) and egg-yolk-based media is recommended (Gordon, 1997), although in this case the insemination time should also be considered (Fernandez-Abella et al., 2003)

4.4.2 Cold shock

Severe changes in temperature are a common feature of semen storage protocols for assisted reproduction, but are not biological traits to which species have become adapted (López-Fernández et al., 2008). In the ram, as in other mammals, there is a loss of semen quality when cooled semen samples are used for assisted reproduction. Cooled semen undergoes a decrease in sperm quality, which includes reduction in motility, destabilisation of sperm membranes and DNA integrity impairment of sperm function (López-Fernández et al., 2008; Muiño-Blanco et al., 2008). It is well known that ram spermatozoa are more sensitive to cold-shock stress than those of other species (Muiño-Blanco et al., 2008). In fact, the ram exhibits a faster DNA degradation under similar conditions than other species studied (Gosálvez et al., 2007). Temperature excursion episodes in spermatozoa are associated with oxidative stress induced by the generation of reactive oxygen species, which promote DNA fragmentation (López-Fernández et al., 2008). Reactive oxygen species, produced by dead spermatozoa during a sperm temperature reduction, give rise to sperm membrane alterations with the subsequent release into the media of free active enzymes. Then, the accumulation of toxic metabolic products and active free enzymes, such as those contained in the acrosome, is higher in the media as spermatozoa disintegrate. This could facilitate intact sperm degradation in an exponential fashion (López-Fernández et al., 2008). This loss of quality, accompanied by a decline in sperm survival in the female reproductive tract, gives rise to a reduction in fertility and increased embryonic loss (Paulenz et al, 2002). In ovine species, ovulation may take place several hours post-insemination (Cheminau et al., 1992; Romano, 2004) and, consequently, the time that a spermatozoon is able to survive after AI is of critical importance to achieve pregnancy. Consequently, obtained semen samples must be used as quickly as possible with the diluents currently available.

4.4.3 Time from semen recovery to dilution

The role of seminal plasma (SP) in mammalian sperm function remains largely a matter of speculation as both inhibitory and stimulating effects have been found (Muiño-Blanco et al., 2008). It has been reported that exposure of ram spermatozoa to seminal plasma causes a reduction of fertility (Dott et al., 1979), although some components of the seminal plasma, such as certain proteins, seem to have the important function of maintaining the stability of the membrane up to the process of capacitation, and are able to protect and repair the cold-shock damage to sperm (Muiño-Blanco et al., 2008). However, in practice, semen dilution in the ram has to be done as soon as possible after recovery to avoid the inhibitory effects of seminal plasma.

4.5 Insemination technique
4.5.1 Semen deposition site

In mammals, establishing an adequate sperm reservoir in the caudal isthmus and utero-tubaric junction is very important after mounting or AI, as spermatozoa may ascend to the fertilisation site from this reservoir. In ovine species, the cervix is the main anatomical and physiological barrier to the ascent of spermatozoa after mounting or AI. This is particularly

relevant after cervical insemination as, in comparison to fresh spermatozoa, a relatively small proportion of the stored cells penetrates the cervical canal and migrates through the uterus of the ewe to the oviducts (Salamon and Maxwell, 2000). Spermatozoa functionally affected during liquid storage may not migrate, or may migrate slowly, and their survival in the female tract is also reduced to about half that of fresh spermatozoa (Salamon and Maxwell, 2000). Attempts to improve the transport of spermatozoa from the posterior cervix to the oviducts of oestrous ewes by prostaglandins added to stored semen have given conflicting results (Maxwell and Salamon, 1993).

In the non-pregnant ewe, the funnel-shaped rings of the cervix, which average around five in number, are not concentrically aligned, and their openings are constricted in most instances to less than 3 mm (King et al, 2004). As explained above, breed is an important determinant of the morphology of the cervix, and that could at least partially explain differences in fertility after cervical AI (Kaabi et al., 2006). Many studies have found a positive correlation between the depth of cervical AI and fertility (Kaabi et al., 2006). In consequence, numerous efforts have been made to develop new methods to deposit the semen as deep as possible into the uterus. Studies based on the use of modified pipettes, or hormones such as oxytocin to dilate the cervical canal, have shown that cervical penetration can be improved. However, fertility results have been very variable (Kaabi, et al., 2006). Special care should be taken to avoid cervical trauma with the catheter during AI, as it has been associated with reductions in pregnancy and lambing rates (Kaabi et al., 2006). Secondary effects of oxytocin may also have an adverse effect on fertility (King, 2004).

4.5.2 Technician

The ability of the inseminator may be another important source of variation of the outcome of sheep AI (Gordon, 1997; Anel et al., 2005). Cervical penetration rates are influenced by operator skill (Eppleston and Maxwell, 1993), and the establishment of training programmes is highly recommended.

4.5.3 Stress around AI

There is some evidence that nutritional or management stress inflicted upon the ewe around AI can markedly reduce fertility by interfering with fertilisation or by increasing early embryo mortality rates (Gordon, 1997). Ewes and rams should be handled with the minimum of disturbance and receive good nutrition around oestrus and the first weeks after AI.

5. Acknowledgements

6. References

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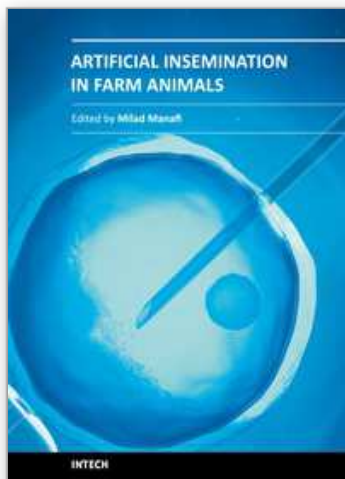
Artificial Insemination in Farm Animals

Edited by Dr. Milad Manafi

Artificial insemination is used instead of natural mating for reproduction purposes and its chief priority is that the desirable characteristics of a bull or other male livestock animal can be passed on more quickly and to more progeny than if that animal is mated with females in a natural fashion. This book contains under one cover 16 chapters of concise, up-to-date information on artificial insemination in buffalos, ewes, pigs, swine, sheep, goats, pigs and dogs. Cryopreservation effect on sperm quality and fertility, new method and diagnostic test in semen analysis, management factors affecting fertility after cervical insemination, factors of non-infectious nature affecting the fertility, fatty acids effects on reproductive performance of ruminants, particularities of bovine artificial insemination, sperm preparation techniques and reproductive endocrinology diseases are described. This book will explain the advantages and disadvantages of using AI, the various methodologies used in different species, and how AI can be used to improve reproductive efficiency in farm animals.

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