SEMEN PRODUCTION AND MANAGEMENT OF RABBIT BUCKS

Cesare Castellini

Dept. of Applied Biology, University of Perugia, Borgo XX Giugno 74, 06121 Perugia, Italy
Corresponding author: cesare@unipg.it

ABSTRACT

The aim of this review was to summarise recent knowledge of the physiology of semen production and management of rabbit bucks. As in other mammals, the spermatogenesis is affected by many environmental factors (temperature, light, age of animals, feeding strategies, health status) as well as by semen handling (dilution, temperature, and condition of storage). Optimal conditions of rearing rabbit bucks can improve the quality of semen permitting to produce additional doses with higher and more stable fertilizing ability. In addition to the use of rabbit as a component of the food chain, rabbit bucks or spermatozoa could be useful as *in vivo* or *in vitro* toxicological models for some chemical compounds. A peculiar aspect of rabbit semen is the large presence of granules produced by prostate gland which, after ejaculation, make contact with spermatozoa. These granules seem implicated in the synchronization between ovulation time and the acrosome reaction. Since in rabbit does the ovulation occurs after several hours from mating, it is highly hypothesisable that during this lag-phase seminal particles contribute to delayed premature capacitation and acrosome reaction and maximize the likelihood that an ovulated egg would meet spermatozoa in the best functional state.

Key words: Semen, Fertility rate, Acrosome reaction, Motility rate, Rabbit.

INTRODUCTION

In commercial rabbit farms, Artificial Insemination (AI) is widely employed and this diffusion has contributed to the increase in knowledge of spermatozoa metabolism and management of rabbit bucks. Many factors affect seminal traits (Boiti *et al*., 2005) and thus it is crucial to define suitable protocols to improve spermatozoa characteristics (Brun *et al.*, 2002 a, b). Hence, it is possible to produce more doses of semen with higher "expected" fertility and with less variability.

Semen is a mixture of spermatozoa, produced by testicles, and seminal plasma secreted at different sites by accessories glands and by the epididymus, which are combined at the time of ejaculation. Seminal plasma also contains other particles of different size which affect the spermatozoa behaviour during the transit along the female reproductive tract (see Figure 1).

Semen evaluation must provide information on the fertilizing ability of spermatozoa. The most relevant parameters correlated with the fertility rate are the number of spermatozoa inseminated and their motility, although the use of a single attribute is not sufficiently accurate to predict the fertilizing ability of the semen (Love *et al.*, 1998; Colenbrander *et al.*, 2003; Lavara *et al.*, 2005). Additional semen traits or composite indexes better predict the fertilizing capacity of spermatozoa. Quinteiro *et al.* (2007), developed a composite index using a multivariate regression approach by entering several parameters of rabbit semen (motility, sperm abnormalities and altered acrosomes), which better predicts the fertilizing ability and the prolificacy of semen samples.

The low correlation between individual semen traits and fertility can also be explained by other reasons such as the use of sperm/AI in excess that masks the effect of several semen quality traits on fertility (Castellini and Lattaioli, 1999; Tardif *et al.*, 1999).

However, variation in the seminal characteristics is known to be affected by many factors (genetic strain, feeding, health status, rearing condition, season, age and collection frequency), thus contributing to the large variability in semen traits (Alvariño, 2000). Furthermore, the complexity of semen evaluation is such that substantial variability among laboratories can be introduced in the evaluation of sperm parameters (sperm counts, motility and morphology; WHO, 1999).

The purpose of this paper was to analyse the main physiological aspects of rabbit semen production and the effect of the management of bucks.

ROLE OF SEMINAL GRANULES IN THE PHYSIOLOGY OF SPERM AFTER EJACULATION

Recent scientific evidence has shown that the particulate fraction of seminal plasma plays an important role in reproductive physiology of several mammal species. These particles are secreted by different accessory glands of the male reproductive apparatus and thus their biochemical composition and function vary from species to species.

Seminal particles have different dimensions: large granules about the sperm head dimension, abundant in rabbit semen (Zaniboni *et al.*, 2004), and small particles described in several other mammalian species (Ronquist *et al.*, 1978; Breitbart and Rubinstein, 1982; Garwal and Vanha Pertulla, 1987; Fornes *et al.*, 1991; El-Hajj *et al.*, 2004).

The electron microscopic view of these particles (Figure 1) shows a prevalent round shape and the presence of clubbed cytoplasmic protrusions with small detaching vesicles. According to Metz *et al.* (1968), rabbit semen granules are not homogeneous and are composed by different populations of vesicles. They show different sizes (0.5-6 μm diameter) and are generally surrounded by a bilaminar membrane containing a scarcely organized electron dense material.

It has been postulated that these particles modulate the capacitation process and acrosome reaction (AR) of spermatozoa (Davis, 1974), kinetics of sperm (Stegmayr and Ronquist 1982; Fabiani *et al.*, 1995), immune-response of female tracts (Kelly *et al.*, 1991; Skibinski *et al.*, 1992; Miodrag *et al.*, 1995; Johansson *et al.*, 2004;), as well as the transit of spermatozoa in the female tract.

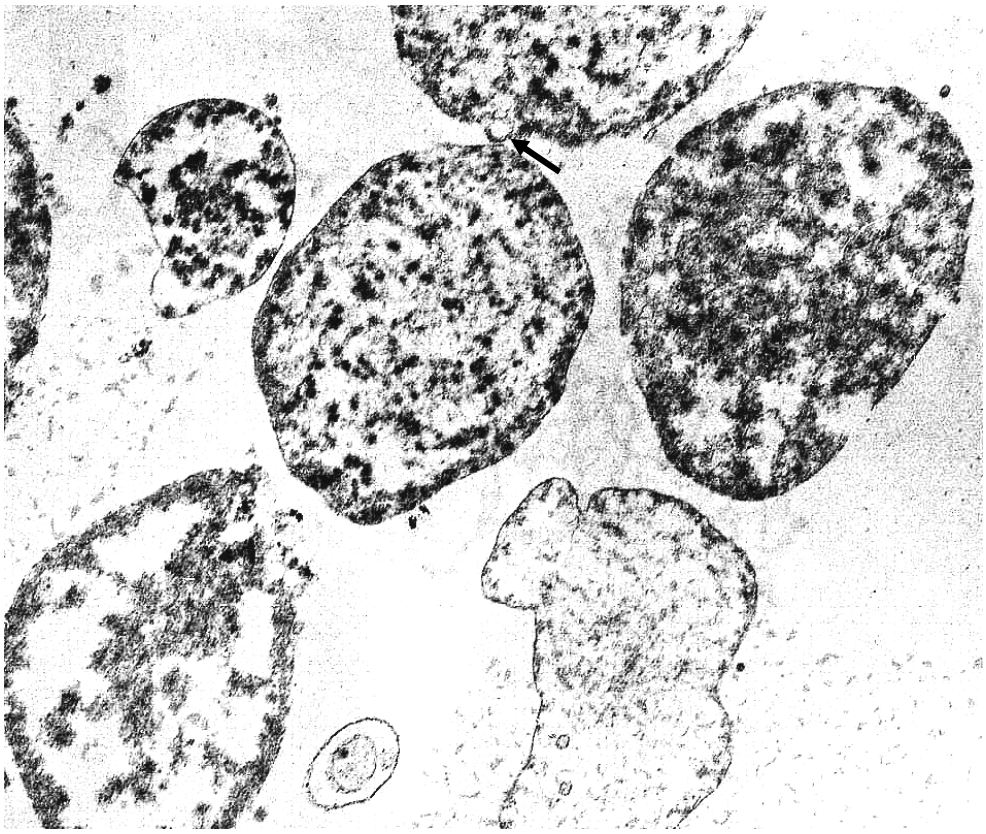

Figure 1: Transmission Electron Microscopy of rabbit seminal granules (original magnification x 11,500); arrow: small detaching particles

These particles, largely found in rabbit semen (450 x 10^6/ml; Castellini *et al.*, 2006b), seem to be mainly involved in the control of capacitation and the acrosome reaction (Table 1). In fact, the presence of seminal granules significantly reduces the response of spermatozoa to *in vitro* inducers of the acrosome reaction and as a result the level of capacitated spermatozoa is almost equal to zero. On the contrary, when granules are eliminated by Percoll ® centrifugation the decapacitative effect is virtually removed.

Table 1: Effect of seminal granules on spontaneous or induced acrosome reaction and capacitated sperm of rabbit (modified from Cardinali *et al.*, 2008)

	Spontaneous acrosome reaction (%)	Induced acrosome reaction (%)	Capacitated (%)
Raw semen	12.5[a]	34.5[a]	22.0[b]
Percoll ® selected sperms	28.5[b]	59.9[b]	31.4[c]
Percoll ® selected sperms + granules	26.2[b]	28.5[a]	2.3[a]
Pooled Standard Error	5.9	4.2	1.9

[a..c] on the same column: $P \leq 0.05$

The decapacitative effect would be ascribed to the release of decapacitating factors by such particles as showed by the lipid composition and the occurrence of ecto-enzymes. Indeed, seminal particles are characterized by large amounts of cholesterol and sphingomyelin (respectively 310 μg/10^9 and 10.20% of fatty acids - Castellini *et al.*, 2006b) and, contrary to most of biological membranes, spermatozoa included, have a molar ratio of phospholipid: cholesterol of approximately 1:2 (Arvidson *et al.*, 1989; Arienti *et al.*, 1997). It is well-known that cholesterol and phospholipids modulate membrane fluidity (Darin-Bennett and White, 1977). In spermatozoa, cholesterol is regarded as the main inhibitor of AR since cholesterol release is required for the activation of a trans-membrane signal transduction leading to sperm capacitation.

Granules and seminal plasma probably act as donors of sterols in order to protect spermatozoa against environmental shock and premature acrosome reaction. According to Cross and Mahasreshti (1997), the inhibitory activity of seminal plasma on AR is mainly contained in such granules and cholesterol is the major inhibitor.

In addition, it should be considered that seminal granules are very rich in tocopherol (38.7 mmol/l more than 50% of semen tocopherol – Mourvaki *et al.*, 2008). The antioxidant activity of tocopherol (Saez *et al.*, 1998), lowering the free radicals in the semen (de Lamirande *et al.*, 1997), could contribute to reducing the responsiveness of spermatozoa to exogenous stimuli.

Nicander *et al.* (1974) demonstrated that these particles are secreted by prostate gland, mainly in the first lobe, called pro-prostate (Figure 2), and their role as modulators of capacitation and AR along the female tract is particularly sound in rabbit. The ovulation reaction in the rabbit female is not spontaneous but is induced by coitus (Jones *et al.*, 1976). Ovulation occurs about 10-16 hours after mating and during this lag-phase rabbit spermatozoa must avoid premature capacitation and AR and seminal particles contribute in delaying this process.

Giojalas *et al.* (2004), comparing human and rabbit spermatozoa, suggest that the timing and duration of the capacitation is programmed according to the egg availability in the oviduct: long in periodic ovulators and short in induced ovulators, such as rabbits. Indeed, Brackett *et al.* (1982) reported that *in vitro* capacitation of raw rabbit semen is long and difficult, whereas Percoll ®-selected spermatozoa (without granules) show a faster rate of capacitation. This circumstance maximizes the possibilities that an ovulated egg would meet spermatozoa in the best functional state.

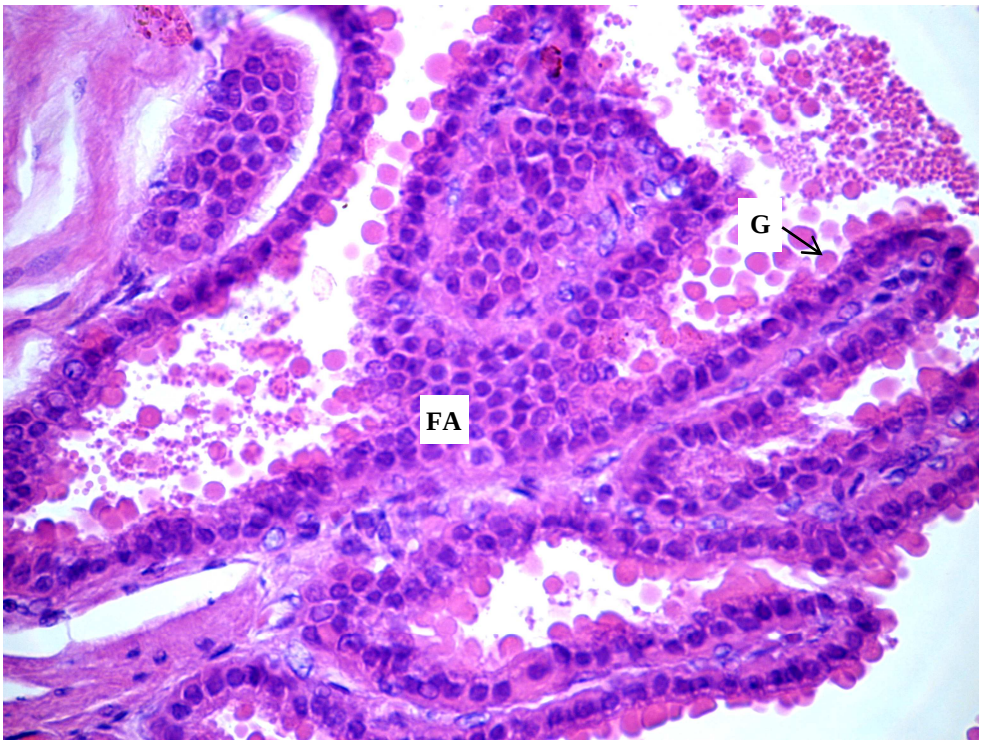

Figure 2: Histology of rabbit pro-prostate (original magnification x 200). Fibrovascular axes (FA) and seminal granules (G) still attached to the secretory cells and free in the gland lumen (from Cardinali *et al.*, 2008)

According to this hypothesis involving seminal plasma, the acrosome is stabilized by two groups of factors: some of them are of epididymus origin (Davis, 1973; Reynolds *et al.*, 1989; Fraser *et al.*, 1990), and are probably effective during spermatozoan maturation, whereas other factors (i.e. seminal particles) may have a crucial role in regulating AR during the passage in the female apparatus and are secreted by other glands of male reproductive apparatus.

FACTORS INFLUENCING SEMEN PRODUCTION

Individual and genetic

The variability of semen characteristics in male rabbits is generally high (Moce *et al.,* 2005); however, the sperm traits of some genetic strains exposed to strict protocols of rearing (light, temperature, feed) and collection frequencies has shown lower variability within and between bucks (Theau-Clement *et al.,* 2003).

Differences between genetic types of bucks have also been found for semen characteristics and fertility. Viudes *et al.* (2004), and Brun *et al.* (2002a, 2004) observed differences in semen characteristics for males from different genetic lines and from crossbred and purebred males. Crossbred sires tended to express a moderate advantage for various semen traits, but when semen from these bucks was used for AI, a negative heterotic effect was observed. Therefore, the use of crossbred males may not provide a major advantage with respect to the use of purebred males from sire lines. These differences could be explained by differences in maternal genetic effects and the existence of heterosis for this trait (Garcia *et al.,* 2006).

Frequency of collection

Collection frequency has an important effect on semen characteristics: two ejaculates collected once a week (with an interval of at least 15 min) allows for the best semen production (Bencheikh, 1995, Moce *et al.,* 2000) both in terms of quality and quantity. Conversely, too long collection frequency (every 14 d) exerts a depressive effect on sperm output probably for the scarce sexual stimulus followed by androgen reduction (Castellini *et al.,* 2006a).

Collection frequency affects not only sperm production but also the concentration of seminal granules: daily collection, in comparison to a collection every week, reduced the spermatozoa and granule concentration even if granules showed a more stable and higher production (Castellini *et al.,* 2006a).

Light

Light length affects the hypothalamus-pituitary axis and consequently hormonal release and spermatozoa production. A daily constant 16L:8D light program increases sperm production (qualitative and quantitative aspects) compared with a shorter light length (8L:16D, Theau-Clément *et al.,* 1994). By contrast, light intensity did not significantly affect semen characteristics (Besenfelder *et al.,* 2004).

Age

Sexual maturity occurs approximately at 5 months (depending on the strain) and semen quality generally decreases in older rabbit bucks. Recently, some authors showed that the sperm chromatin structure of the semen of rabbits between 5 and 28 months of age significantly changed. Changes in chromatin structure suggested a relatively high stability of sperm chromatin in the rabbit. The lowest percentage of sperm with damaged chromatin (1.7-2.4%) was observed between 6 and 16 months of age. Decreased sperm chromatin stability was found in ejaculates taken from male rabbits less than 5 months and more than 20 months of age (Gogol *et al.,* 2002).

Spermatozoa of aged animals show less stable membranes and they seem more vulnerable to dietary deficiency of polyunsaturated fatty acids (PUFA; Castellini *et al.,* 2003a).

Health status

It is widely known that inflammation of the male reproductive apparatus (O'Bryan *et al.,* 2000) worsens various testicle functions and seminal characteristics by affecting biosynthesis of pro-

inflammatory eicosanoids (prostaglandins and leukotriens) and cytokines (Knapp, 1990).

A high concentration of leukocytes during spermatogenesis or after ejaculation caused by inflammation/infection can deeply reduce the integrity of acrosome by increasing free radical production. Bucks' health has to be regularly controlled mainly in aged animals.

Feeding protocols

Regarding the quantity of feed that should be administered to rabbit bucks, Luzi *et al.* (1996) showed that a restricted dietary protocol reduces *libido* and some seminal traits. However, the most important factor is not the amount of diet furnished but its chemical characteristics.

Specific recommendations for rabbit bucks are not available (de Blas and Wiseman, 1998), and only some specific requirements have been established.

Crude protein
Diets with more than 15% of crude protein are recommended to assure suitable sperm production (Nizza *et al.*, 2000).

Fat
More critical than the total amount of fat is a balanced fatty acid composition (Wesley, 1998). In mammalian spermatozoa, a very high amount of lipids are PUFA of n-3 and n-6 series (Apel-Paz *et al.*, 2003), which are associated with the membrane fluidity and its competence. Animal species are not able to synthesize essential PUFA (C18:2n-6 or C18:3n-3) and the diet has to provide adequate amounts of these fatty acids in the form of precursors (C18) or elongated ($C \geq 20$) fatty acids.

Our previous research has shown that dietary addition of PUFA n-3 modified several traits of rabbit spermatozoa (Table 2: Castellini *et al.*, 2003b; Castellini *et al.*, 2004). Relevant modifications regarding the motility and the kinetic traits of sperm cells (curvilinear velocity and lateral head displacement) is probably due to higher membrane elasticity in spermatozoa of n-3 supplemented group. The best results occurred when 2% fish or 20% flaxseed were added to the diets (Castellini *et al.*, 2005).

Table 2: Effect of dietary supplementation with n-3 fatty acids on semen traits of rabbits (modified from Castellini *et al.*, 2005)

Treatment		Control	Fish 2%	Flaxseed 5%	Flaxseed 20%
Concentration	$n.x10^6$ ml	353^a	410^b	389^{ab}	493^b
Motile cells	%	69.9^a	76.6^b	72.9^{ab}	79.0^b
Curvilinear velocity	μm/sec	108^a	123^b	115^a	130^b
Linearity	%	48.5^b	42.6^a	47.1^b	43.5^a
Lateral head displacement	μm	3.62^a	4.09^b	3.87^{ab}	4.34^b
Spontaneous acrosome reaction	%	13.5	13.5	15.6	14.0
Induced acrosome reaction	%	31.0	36.6	35.4	37.7
Capacitated sperm	%	17.5^a	23.1^b	19.8^{ab}	23.7^b

$^{a..c}$ on the same raw: $P \leq 0.05$

On the contrary, high levels of cholesterol in the diet alter the metabolism of Sertoli cells (Yamamoto *et al.*, 1999) and the normal process of spermatogenesis (Mann and Lutwak-Mann, 1981).

Antioxidant protection
The high unsaturation levels of spermatozoa membrane render these cells very susceptible to peroxidation (de Lamirande *et al.*, 1997), which degrades membrane structure, sperm metabolism and DNA integrity (Jones *et al.*, 1979).

The more common way to increase the antioxidant stability of semen is to fortify diets with antioxidant molecules. Alpha-tocopherol (α-T) is retained as one of the most powerful antioxidants

and high dietary levels of α-T, mainly if associated with vitamin C (Castellini *et al.,* 2000a, c; Castellini *et al.,* 2001b; Castellini *et al.,* 2003b), is effective in limiting the oxidative damage of semen.

However, the bioavailability of α-T in blood and in semen is non-linear: rabbit bucks fed 200 mg/kg α-T acetate showed a proportional increase after only 1 week of administration (Castellini *et al.,* 2002; Castellini *et al.,* 2006c). Studies using deuterated α-T demonstrated that newly administered a-T rapidly displaced 'old' α-T in plasma rather than proportionately increasing the total plasma α-T level (Traber *et al.,* 2001).

It should be noted that α-T in rabbits has a lower bioavailability than in humans (Galli *et al.,* 2001) probably because of the low fat, high fiber of rabbit diet which limits vitamin E absorption (Iuliano *et al.,* 2001).

To decide the amount of α-T to be used as well as the way in which it should be administered, a suitable tool was the analysis of the major α-T catabolite (Carboxyethoxychroman: CEHC) in rabbit urine. Rabbit bucks supplemented with a mono-dose of α-T acetate (800 and 1600 mg/animal) after 24 hours showed large differences in CEHC concentration according to the dose administered (Figure 3) but at the same plasma level of α-T. After 72 hours the CEHC level in the urine was the same without any increase in the α-T plasma level.

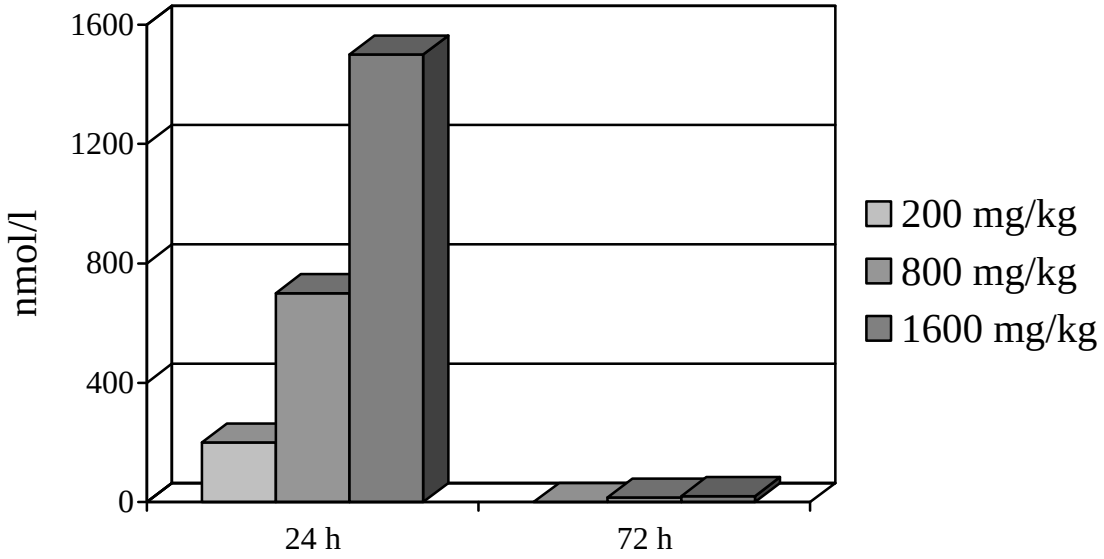

Figure 3: Carboxyethoxychroman (CEHC) levels in urine of rabbit bucks 24–72 h after α-T administration

Considered together, these findings may be used to define the amount of α-T to be used as well as the way in which it should be administered. Doses that exceed the capability of saturable enzymes and plasma transporters have no effect on the α-T bioavailability because α-T in excess is eliminated. Therefore, continuous supplementation not exceeding the plasma α-T threshold (absence of CEHC) is more efficient than the use of single mega-dose (Castellini *et al.,* 2007a).

In semen, The trend of α-T is even more complex than in plasma. As in other species, the standard level of α-T in rabbit semen is relatively low (0.2-0.9 mmol/l; Marin-Guzman *et al.,* 1997). To increase this level, a long lag-phase of α-T accumulation occurs according to the time needed for reaching the site of action and then further time is needed for spermatozoa to differentiate and mature (about 45–50 days). When dietary supplementation is extended beyond that period, the α-T

concentration in testis and spermatozoa increases, reaching a steady state after about 3 months (Castellini *et al.,* 2006c). Although the increase of α-T in semen is not particularly high, its oxidative stability is greatly enhanced because antioxidant efficiency of α-T mainly depends on its incorporation into the spermatozoa membranes. This was also confirmed by the fact that the antioxidant protection of semen is not greatly reduced after dilution (Castellini *et al.,* 2000a).

Even if α-T is the main natural form of Vitamin E existing in the body, eight other molecules are included in the definition of vitamin E, α–, β–, γ– and δ-tocopherol whose amount and role are largely unknown. The higher level of α-T compared to other homologues was probably due to the high concentration of this compound in the standard diet (90%). Such high amounts of α-T compromise the access of the other isoforms to saturable lipoproteins, binding proteins and enzymes that implicated in their transport, distribution and metabolism, and this may explain the low amounts of these compounds in semen.

According to Mourvaki *et al.* (2008), α-T, β-T, γ-T and δ-T are not homogeneously distributed within rabbit semen fractions (spermatozoa, seminal plasma and granules). Alpha-T is the main lipid antioxidant in all fractions, while γ-T, β-T and δ-T are more abundant in germ cells and seminal plasma, respectively. Differences in the chemical structure might account for the differential distribution of the minor tocopherol isoforms within fractions. More polar tocopherols (δ-T) have a higher affinity for aqueous seminal plasma, while less polar tocopherols (α-T > β-T > γ-T ordered by non-polarity) have a higher affinity for the lipid-rich cell membrane and seminal granules.

This antioxidant protection is essential after semen storage or when diets have a relevant amount of PUFA (Figure 4). When diets are fortified with different sources (fish and flaxseed) and levels (2-20%) of PUFA, the antioxidant stability of semen decreases. In addition, these dietary protocols determine a higher susceptibility of spermatozoa to capacitation and the acrosome reaction (Castellini *et al.,* 2001a; 2005).

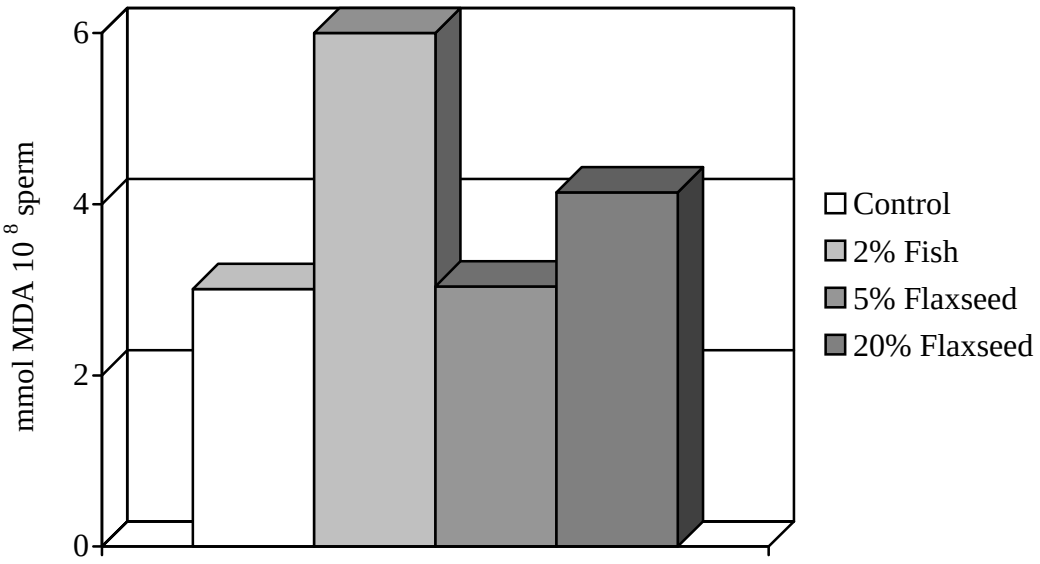

Figure 4: Effect of dietary supplementation with n-3 fatty acids on antioxidant stability of rabbit semen

SEMEN COLLECTION AND HANDLING

Some authors reported that a previous stimulation of the buck increases sperm concentration. For this aim, a doe can be put on the top of the cage for few minutes.

The type of artificial vagina influences the adaptation of buck to the collection. An artificial vagina with a wider collection hole increases the number of bucks adapted to the collection (Dal Bosco *et al.*, 1996). At each collection time, a sterile artificial vagina should be used. Indeed, considerable bacterial contaminants come from the environment, and it is important to hygienically collect the semen sample (Mercier and Rideaud, 1990).

As a general rule, during semen handling, any shock of sample (temperature, chemical, oxygen) may be reduced to avoid reduced fertility (Boiti *et al.*, 2005).

Within 5 min from collection, semen should be diluted (1/2-1/5) with a buffer medium at the same temperature to avoid heat or cold shocks. Generally, all the semen handling has a negative effect on semen traits.

STORAGE CONDITIONS

Temperature
Regarding the storage temperature, generally 15-18 °C represents a good condition to store rabbit semen. Nevertheless, optimal temperature could depend on the extender used.

Extenders
One important factor to have good survival rate of sperm is the medium used in semen dilution and the dilution rate. The comparison between different media has shown that any physiologic buffered saline solution is adequate for very short storage period. However, for longer storage there are differences in the ability to support sperm survival (Seed *et al.*, 1996). Tris-buffer is enough to allow storage from 24-48h.

The high dilution rate (more than 1/100; Castellini *et al.*, 2000b) has a detrimental effect on motility and the excessive dilution of seminal plasma, which in rabbit plays a relevant role (Minelli *et al.*, 2001), which reduces kinetic characteristics of spermatozoa.

Incubation
In accordance to specific demands of semen for further laboratory analysis or for AI, an adequate procedure has to be chosen to maintain vitality and physiological capability of fertilisation over a defined time interval. Thus, semen has to be diluted or spermatozoa have to be separated and put into a final medium. For semen dilution as well as for the spermatozoa containing medium, optimal conditions must be assured by selecting buffers that are adapted to the reproduction organs; CO_2 (5%) incubators preferentially will support long term spermatozoa vitality by simultaneously reducing excessive contact with oxygen.

RABBIT SEMEN AS MODEL FOR TOXICOLOGICAL OR METABOLIC STUDIES

Exposure to some chemical compounds can alter semen quality and fertility. Since the prevalence of human infertility in industrialized countries has increased over the past decades, the assessment of the risk to the human reproductive system associated with exposure to compounds due to pollution is of major concern.

Mice are widely used for toxicological studies because of the massive reported information on its development and functions, along with responses to many toxicants. Even for rabbit, the main

physiological traits have been studied, both as a laboratory animal and as a component of the food chain. The rabbit is the smallest laboratory and least expensive animal model, wherein almost all the reproductive and toxicological endpoints of humans can be measured. The rabbit semen can be easily collected by artificial vagina and the fertility of sperm tested (Foote and Carney, 2000; Foote, 2002).

For *in vivo* models, a very useful method could be the use the spermatozoon as a toxicological target. The mature spermatozoon is a cell that is eliminated of almost all the normal biological functions but retains those dedicated to provide motility and the fertilisation ability. Damages to one of such structures are easily detectable because the ejaculated spermatozoon is unable to activate a repairing process. Following these considerations, it is sound to use the spermatozoon as a good target for metabolic (Kamp *et al.*, 1996) and toxicological studies (D'Cruz *et al.*, 2000).

According to such assumptions, some authors (Young *et al.*, 1992; Foote, 2002) hypothesized the use of rabbit spermatozoa as a model for toxicological studies and proposed motion-based indices as toxicological endpoints. For several chemicals, motion-based endpoints of spermatozoa may be useful for the *in vitro* assessment of chemical cytotoxicity.

It is quite evident that to develop a repeatable *in vitro* spermatozoa model for *in vitro* toxicity tests, different preliminary steps of standardization are required. Accordingly, the variability factors are reduced (Seed *et al.*, 1996) and the discriminating power of the model increased (Williams *et al.*, 1990).

Renieri *et al.* (2002) and Sartini *et al.* (2006), within a research program of the European Centre of the Validation of Alternative Methods, developed an *in vitro* rabbit spermatozoa model of spermiotoxicity for some metal ions. It involves the use of integrated strategies based on data derived from human and animal studies, as well as *in vitro* toxicity test, which also lead to a reduction in the use of laboratory animals that is very advantageous for ethical and economic reasons.

It appears that some endpoints of this rabbit spermatozoa model involving motility rate and the integrity of the acrosome are very sensitive traits for detecting metal ions damages and could be useful in assessing toxicity.

ACKNOWLEDGEMENTS

Acknowledgements: Some results referred in this review are partly funded by PRIN 2005 prot. 2005070702_004.

REFERENCES

Alvariño J.M.R. 2000. Reproductive performance of male rabbits. *In Proc. 7th World Rabbit Congress, July 2000, Valencia, Spain, Vol. A, 13-35.*

Apel-Paz M., Vanderlick T.K., Chandra N., Doncel F.G. 2003. A hierarchy of lipid constructs for the sperm plasma membrane. *Biochemical Biophysical Research Communications, 309, 724-732.*

Arienti G., Carlini E., Palmerini C.A. 1997. Fusion of human sperm to prostasomes at acidic pH. *J. Membr. Biol., 155, 89-94.*

Arvidson G., Ronquist G., Wikander G., Ojteg A.C. 1989. Human prostasome membranes exhibit very high cholesterol/phospholipids ratios yielding high molecular ordering. *Biochimica et Biophysica Acta, 984, 167-173.*

Bencheikh N. 1995. Effet de la fréquence de collecte de la semence sur les caractéristiques du sperme. *Ann. Zoot., 44, 263-279.*

Besenfelder U., Theau-Clément M., Sabbioni E., Castellini C., Renieri T., Havlicek V., Huber T., Wetscher F., Mösslacher G., Brem G. 2004. Effects of different light intensities on quality of spermatozoa in rabbits. *World Rabbit Science, 12, 227-234.*

Boiti C., Castellini C., Besenfelder U., Theau-Clément M., Liguori L., Renieri T., Pizzi F. 2005. Guidelines for the handling of rabbit bucks and semen. *World Rabbit Science, 13, 71-91.*

Brackett B.C., Bousquet D., Dressel M.A. 1982. In Vitro sperm capacitation and in vitro fertilization with normal development in the rabbit. *J. Androl., 3, 402-411.*

Breitbart H., Rubinstein S. 1982. Characterization of Mg2+ and Ca2+-ATPase activity in membrane vesicles from ejaculated ram seminal plasma. *Arch. Androl., 9, 147-157.*

Brun J.M., Theau-Clément M., Bolet G. 2002a. Evidence for heterosis and maternal effects on rabbit semen characteristics. *Anim. Res., 51, 433-442.*

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

Brun J.M., Theau-Clement M., Larzul C., Falieres J., Saleil G. 2004. Semen production in two rabbit lines divergently selected for 63-d body weight. *In Proc. 8th World Rabbit Congress, September 2004, Puebla, Mexico, 238-244.*

Cardinali R., Dal Bosco A., Mourvaki E., Del Vecchio M.T., Sartini B., Renieri T., Lasagna E., Castellini C. 2008. Rabbit semen particles: secretion pattern and main effect in the sperm functions *J. Sub. Cytol. Pathol. (In press).*

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

Castellini C., Lattaioli P., Bernardini M., Dal Bosco A. 2000a. Effect of dietary α-tocopheryl acetate and ascorbic acid on rabbit semen storage at 5 °C. *Theriogenology, 54, 523-533.*

Castellini C., Lattaioli P., Minelli A., Moroni M. 2000b. Effect of seminal plasma on the characteristics and fertility of rabbit spermatozoa. *Anim. Reprod. Sci., 63, 275-282.*

Castellini C., Dal Bosco A., Bernardini M., 2000c. Effect of dietary alfa-tocopheryl acetate and ascorbic acid: vitamin content and oxidation status of rabbit semen. *In Proc. 7th World Rabbit Congress, July 2000, Valencia, Spain, Vol. A, 105-110.*

Castellini C., Dal Bosco A., Lattaioli P., Allegrucci C., Bernardini M. 2001a. Lipid profile and oxidative status of spermatozoa of rabbit fed high levels of n-3 fatty acids. *In Proc. XIV ASPA Congress, June 2001, Firenze, Italy, 424-426.*

Castellini C., Lattaioli P., Dal Bosco A., Liguori L. 2001b. Effet de l'addition des vitamines C et E sur les paramètres de la semence de lapin après congélation. *In Proc. 9émes Journées Recherche Cunicole, November 2001, Paris, France, 151-154.*

Castellini C., Lattaioli P., Dal Bosco A., Beghelli D. 2002. Effect of supranutritional level of dietary a-tocopheryl acetate and selenium on rabbit semen. *Theriogenology, 58, 1723-1732.*

Castellini C., Boiti C., Dal Bosco A., Lattaioli P., Zampini D. 2003a. Effet de la supplementation en acides gras n-3 et vitamine E sur les caractéristiques de la semence de lapins d'âges différentes. *In Proc. 10émes Journées Recherche Cunicole, November 2003, Paris, France, 77-80.*

Castellini C., Lattaioli P., Dal Bosco A., Minelli A., Mugnai C. 2003b. Oxidative status and semen characteristics of rabbit buck as affected by dietary vitamin E, C and n-3 fatty acids. *Repr. Nutr. Dev., 43, 91-103.*

Castellini C., Dal Bosco A., Cardinali R., Mugnai C. 2004. Effect of dietary α-linolenic acid on semen characteristics of rabbit bucks. *In Proc. 8th World Rabbit Congress, September 2004, Puebla, Mexico, 245-250.*

Castellini C., Cardinali R., Lattaioli P., Dal Bosco A. 2005. Comparison of different dietary sources of PUFA n-3 on semen characteristics of rabbit bucks. *Repr. Dom. Anim. (Abstr. 386), 180.*

Castellini C., Lattaioli P., Cardinali R., Dal Bosco A. 2006a. Effect of collection rhythm on spermatozoa and droplet concentration of rabbit semen. *World Rabbit Science, 14, 101-106.*

Castellini C., Cardinali R., Dal Bosco A., Minelli A., Camici O. 2006b. Lipid composition of the main fractions of rabbit semen. *Theriogenology, 166, 703-712.*

Castellini C., Dal Bosco A., Cardinali R., Mourvaki E. 2006c. Effect of dietary vitamin E on tissue distribution and semen quality of rabbit bucks. *In Proc. 10th Jubilee Annual Conference of the ESDAR, September 2006, Potoroz-Lipica, Slovenia, 326.*

Castellini C., Mourvaki E., Dal Bosco A., Galli F. 2007a. Vitamin E biochemistry and function, a case study in male rabbit. *Reprod. Domest. Anim., 42, 248-256.*

Colenbrander B., Gadella B.M., Stout T.A.E. 2003. The predictive value of semen analysis in the evaluation of stallion fertility. *Reprod. Domest. Anim., 38, 305-311.*

Cross N.L., Mahasreshti P. 1997. Proteasome fraction of human seminal plasma prevents sperm from becoming acrosomally responsive to the agonist progesterone. *Archiv. of Andrology, 39, 39-44.*

Dal Bosco A., Scuota S., Castellini C., Cenci T. 1996. Study of an artificial vagina to reduce the microbial contamination of rabbit semen. *World Rabbit Science, 4, 201-204.*

D'Cruz O.J., Vassilev A., Uckun F.M. 2000. Evaluation of boar sperm as a model system to study the mechanism of spermicidal activity of vanadocenes. *Biochemical Biophysical Research Communications, 270, 826-830.*

Darin-Bennett A., White I.G. 1977. Influence of the cholesterol content of mammalian spermatozoa on susceptibility to cold-shock. *Cryobiology, 14, 466-470.*

Davis B.K. 1973. Occurrence of vesicles in rabbit seminal plasma. *Experientia, 29, 1484-1487.*

Davis B.K. 1974. Decapacitation and recapacitation of rabbit spermatozoa treated with membrane vesicles from seminal plasma. *J. Reprod. Fertil., 41, 241-244.*

de Blas C. and Wiseman J. 1998. *In: De Blas C., Wiseman J. (Eds). The Nutrition of the Rabbit. CABI Publishing. CAB International, Wallingford Oxon, UK, 241-253.*

de Lamirande E., Jiang H., Zini A., Kodama H., Gagnon C. 1997. Reactive oxygen species and sperm physiology. *Rev. Reproduction, 2, 48-54.*

El-Hajj Ghaoui R., Thomson P.C., Evans G., Maxwell W.M. 2004. Characterization and localization of membrane vesicles in ejaculate fractions from the ram, boar and stallion. *Reprod. Domestic. Anim., 39, 173-180.*

Fabiani R., Johansson L., Lundkvist O., Ronquist G. 1995. Prolongation and improvement of prostasome promotive effect on sperm forward motility. *European J. of Obstetrics Gynecology and Reproductive Biology, 58, 191-198.*

Foote R.H. 2002. Effects of metronidazole, ipronidazole, and dibromochloropropane on rabbit and human sperm motility and fertility. *Reprod. Toxicol., 16, 749-755.*

Foote R.H., Carney E.W. 2000. The rabbit as a model for reproductive and developmental toxicity studies. *Reprod. Toxicol., 14, 477-493.*

Fornes M.W., Barbieri A., Sosa M.A., Bertini F. 1991. First observations on enzymatic activity and protein content of vesicles separated from rat epididymal fluid. *Andrologia, 23, 347-351.*

Fraser L.R., Harrison R.A., Herod J.E. 1990. Characterization of a decapacitation factor associated with epididymal mouse spermatozoa. *J. Reprod. Fert., 89, 135-148.*

Galli F., Lee R., Dunster C., Atkinson J., Floridi A., Kelly F.J. 2001. Gamma-Tocopherol metabolism and its relationship with alpha-tocopherol in humans: a stable isotope supplementation study. *Biofactors, 15, 65-69.*

Garcia M., Piles M., Sanchez J., Rafel O., Ramon J., Piles M. 2006. Heterosis and maternal genetic effects on semen quality traits of rabbits. *Livest. Sci., 104, 233-243.*

Garwal Y., Vanha Pertulla T. 1987. Effect of secretory particles in bovine seminal vesicle secretion on sperm motility and acrosome reaction. *J. Reprod. Fert., 79, 409-419.*

Giojalas L., Rovasio R., Fabro G., Gakamsky A., Eisenbach M. 2004. Timing of sperm capacitation appears to be programmed according to egg availability in the female genital tract. *Fert. Ster., 82, 247-249.*

Gogol P., Bochenek M., Smora Z. 2002. Effect of rabbit age on sperm chromatin structure. *Reprod. Dom. Anim., 37, 92-95.*

Iuliano L., Micheletta F., Maranghi M., Frati G., Diczfalusy U., Violi F. 2001. Bioavailability of vitamin E as function of food intake in healthy subjects: effects on plasma peroxide scavenging activity and cholesterol-oxidation products. *Arterioscler. Thromb. Vasc. Biol., 21, 34-37.*

Johansson M., Bromfield J.J., Jasper M.J., Robertson S.A. 2004. Semen activates the female immune response during early pregnancy in mice. *Immunology, 112, 290-300.*

Jones E.F., Bain J.B., Odell WD. 1976. Postcoital luteinizing hormone release in male and female rabbits as determined by radioimmunoassay. *Fertil Steril., 27, 848-852.*

Jones R., Mann T., Sherins R. 1979. Peroxidative breakdown of phospholipids in human spermatozoa, spermicidal, properties of fatty acid peroxides, and protective action of seminal plasma. *Fertil. Steril., 31, 531-537.*

Kelly R.W., Holland P., Skibinski G., Harrison C., McMillan L., Hargreave T., James K. 1991. Extracellular organelles (prostasomes) are immunosuppressive components of human semen. *Clin. Exp. Immunol., 86, 550-556.*

Knapp H.R. 1990. Prostaglandins in human semen during fish oil ingestion, evidence for in vivo cyclooxygenase inhibition and appearance of novel trienoic compounds. *Prostaglandins, 39, 407-423.*

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

Love C.C., Kenney R.M. 1998. The relationship of increased susceptibility of sperm DNA to denaturation and fertility in the stallion. *Theriogenology, 50, 955-972.*

Luzi F., Maertens L., Mijen P., Pizzi F. 1996. Effect of feeding level and dietary protein on libido and semen characteristics of bucks. *In Proc. 6th World Rabbit Congress, July 1996, Toulouse, France, vol. 2, 87-92.*

Mann T., Lutwak-Mann C. 1981. Biochemistry of seminal plasma and male accessory fluids; application to andrological problems. *In: Mann T., Lutwak-Mann C. (Eds.), Male Reproductive Function and Semen. Springer-Verlag, Berlin, Germany, 269-326.*

Marin-Guzman J., Mahan D.C., Chung Y.K., Pate J.L., Pope W.F. 1997. Effects of dietary selenium and vitamin E on boar performance and tissue responses, semen quality, and subsequent fertilization rates in mature gilts. *J. Anim. Sci., 75, 2994-3003.*

Mercier P., Rideaud P. 1990. Bactériologie du sperme frais de lapin. *Prod. Anim., 3, 215-221.*

Metz C.B., Hinsch G.W., Anika J.L. 1968. Ultrastructure and antigens of particles from rabbit semen. *J. Reprod. Fertil., 17, 195-198.*

Minelli A., Moroni M., Castellini C. 2001. Isolation of the IGF-I protein complex from rabbit seminal plasma: effects on sperm mobility and viability. *J. Experimental Zoology, 290, 279-290.*

Minelli A., Liguori L., Bellezza I., Renieri T., Castellini C. 2003. Effects of diadenosine polyphosphates and seminal fluid vesicles on rabbit sperm cells. *Reproduction, 125, 827-835.*

Miodrag L., Skibinski G., Rodney K.W., Keith J. 1995. Immunomodulatory effects of extracellular secretory vesicles isolated from bovine semen. *Veterinary Immunology and Immunopatology, 44, 237-250.*

Mocé E., Lavara R., Lavara F., Vicente J.S. 2000 . Effect of reproductive rhythm on seminal parameters from a rabbit line selected with high growth rate. *In Proc. 7th World Rabbit Congress, July 2000, Valencia, Spain, Vol. A, 197-201.*

Mocé E., Vicente J.s, Lavara R., Viudes De Castro M.P., Lopez M, Bolet G. 2005. Characteristics of fresh semen from eight rabbit breeds. *Reprod. Domest. Anim., 40, 388-398.*

Mourvaki E., Collodel G., Moretti E., Cosci I., Castellini C. 2008. Distribution of alpha-, gamma (+beta)- and delta-tocopherol in the seminal plasma, spermatozoa and seminal vesicles of rabbit. *Andrologia (In press).*

Nicander L., Ploen L., Larson M. 1974. Specific apocrine secretion in the anterior lobe of the prostate gland of rabbits. *Cell. Tiss. Res., 151, 69-79.*

Nizza A., Di Meo C., Taranto S. 2000. Influence of dietary protein content on libido and semen characteristics bucks. *In Proc. 7th World Rabbit Congress, July 2000, Valencia, Spain, Vol. A, 217-224.*

O'Bryan M.K., Schlatt S., Phillips D.J., De Kretser D.M., Hedger M.P. 2000. Bacterial lipopolysaccharide-induced inflammation compromises testicular function at multiple levels in vivo. *Endocrinol., 141, 238-246.*

Quintero-Moreno A., Rigaul T., Rodríguez-Gil JE. 2007. Multivariate cluster analysis regression procedures as tools to identify motile sperm subpopulations in rabbit semen and to predict semen fertility and litter size. *Reprod. Dom. Anim., 42, 312-319.*

Renieri T., Sabbioni E., Fortaner S., Castellini C., Theu-Clément M., Besenfelder U. 2002. Development of an *in vitro* rabbit sperm model of metal-induced infertility and spermiotoxicity for regulatory purpose. *In Proc. 9th International Symposium on Spermatology, October 2002, Cape Town, South Africa, 279-282.*

Reynolds A.B., Thomas T.S., Wilson W.L., Oliphant G. 1989. Concentration of acrosome stabilizing factor (ASF) in rabbit epididymal fluid and species specificity of anti-ASF antibodies. *Biol. Repr., 40, 673-680.*

Robert M., Gagnon C. 1996. Purification and characterization of the active precursor of a human sperm motility inhibitor secreted by the seminal vesicles: identity with semenogelin. *Biol. Repr., 55, 813-821.*

Ronquist G., Brody I., Gottfries A., Stegmayr B. 1978. An Mg^{2+} and Ca^{2+} -stimulate adenosine triphosphatase in human prostatic fluid. *Andrologia, 10, 261-272 (part I) and 427-433 (part II).*

Saez F., Motta C., Boucher D., Grizard D. 1998. Antioxidant capacity of prostasomes in human sermen. *Mol. Hum. Reprod., 4, 667-672.*

Sartini B., Besenfelder U., Castellini C., Collodel G., Sabbioni E., Theau-Clement M., Renieri T. 2006. Metal ions effect on rabbit sperm cells. *In Proc. 10th International Symposium on Spermatology, September 2006, El Escorial, Spain, 8-11.*

Seed J., Chapin R.E., Clegg E.D., Dostal L.A., Foote R.H., Hurtt M.E., Klinefelter G.R., Makris S.L., Perreaultys S.D., Schrader S., Seyler D., Sparando R., Treine K.A., Veeramacheneni D.N.R., Wise L.D. 1996. Method for the assessing sperm motility, morphology, and counts in the rat, rabbit, and dog: a consensus report. *Reprod. Toxicol., 10, 237-244.*

Skibinski G., Kelly R.W., Harkiss D., James K. 1992. Immunosuppression by human seminal plasma extracellular organelles (prostasomes) modulate activity of phagocytic cells. *Am. J. Reprod. Immunol., 28, 97-103.*

Stegmayr B, Ronquist G. 1982. Stimulation of sperm progressive motility by organelles in human seminal plasma. *Scand. J. Urol. Nephrol., 16, 85-90.*

Tardif S., Laforest J.P., Cormier N., Bailey L. 1999. The importance of porcine sperm parameters on fertility in vivo. *Theriogenology, 52, 447-459.*

Theau-Clément M., Michel N., Esparbié J., Bolet G. 1994. Effect of artificial photoperiods on sexual behaviour and sperm output in the rabbit. *Anim. Sci., 60, 143-149.*

Theau-Clément M., Brun J.M., Sabbioni E., Castellini C., Renieri T., Besenfelder U., Falières J., Esparbié J., Saleil G. 2003. Comparison de la production spermatique de trois souches de lapins: moyennes et variabilités. *In Proc. 10èmes Journées Recherche Cunicole, November 2003, Paris, France, 81-84.*

Traber M.G., Winklhofer-Roob B.M., Roob J.M., Khoschsorur G., Aigner R., Cross C., Ramakrishnan R., Brigelius-Flohe R. 2001. Vitamin E kinetics in smokers and nonsmokers. *Free Radic. Biol. Med., 31, 1368-1374.*

Viudes de Castro M.P., Marco-Jiménez F., Vicente J.S., Navarro E., Lavara R., Mocé E. 2004. Sperm kinetic parameters and differences in seminal plasma composition among two rabbit lines. *In Proc. 8th Annual Conf. European Society of Domestic Animal Reproduction. Reproduction in Domestic Animals 394. 266 Abstract (P13). Warsaw Agricultural University, Poland.*

Wesley A.J. (1998). Immunonutrition: the role of ω-3 fatty acids. *Nutrition, 14, 627-633.*

WHO (1999). Laboratory manual for the examination of human semen and sperm-cervical mucus interaction. *In: 4th ed. Cambridge University Press, Cambridge, UK.*

Williams J., Gladen B.C., Schrader S.M., Turner T.W., Phelps J.L., Chapin R.E. 1990. Semen analysis and fertility assessment in rabbits: statistical power and design considerations for toxicology studies. *Fundamental Applied Toxicology, 15, 651-665.*

Yamamoto Y., Shimamamoto K., Sofukitis N., Miyagawa I. 1999. Effect of hypercholesterolemia on Leydig and Sertoli cell secretory function and the overall sperm fertilizing capacity in the rabbit. *Human Reprod., 14, 1516-1521.*

Young R.J., Bodt B.A., Iturralde T.G, Starke W.C. 1992. Automated analysis of rabbit sperm motility and the effect of chemicals on sperm motion parameters. *Mol. Reprod. Develop., 33, 347-356.*

Zaniboni L., Gliozzi T., Maldjian A., Luzi F., Cerolini S. 2004. Fatty acid and tocopherol composition of semen components in the rabbit. *In Proc. 8th World Rabbit Congress, September 2004, Puebla, Mexico, 365-370.*