Available online at www.sciencedirect.com
ScienceDirect
Theriogenology 68 (2007) 308–315
Theriogenology
www.theriojournal.com

Influence of the male on embryo quality

P.J. Chenoweth *

School of Agricultural and Veterinary Sciences, Charles Sturt University, P.O. Box 588, Wagg Wagga, New South Wales 2650, Australia

Abstract

Early pregnancy failure or loss (EPL) represents a major source of wastage and inefficiency in livestock production systems. Although successful embryo development is dependent upon genetic and epigenetic contributions from both the male and female, potential adverse male affects on embryo quality and development are probably often underestimated. Of adverse male effects which have been identified, those associated with sperm and semen ''quality'' have been best characterized. In turn, although many factors can adversely impact semen quality, the mechanisms involved are relatively few. This presents opportunities for identifying biological markers for spermatogenic damage, as well as protective measures.

Keywords: Male-factor infertility; Embryo quality; Embryonic death; Sperm; Semen

1. Introduction

Early pregnancy failure or loss (EPL) is a major source of wastage and inefficiency in livestock production systems. In general, mammals incur high and variable rates of EPL [1], with most loss occurring early in development. The scope of this loss is directly associated with poor developmental competence of embryos, with less than half of fertilized human and bovine oocytes reaching the blastocyst stage [1]. Of those that do reach blastocyst stage, many do not subsequently implant or attach, and a further subset are lost in early pregnancy for reasons that are largely obscure [2].

The reasons for this high failure rate have not been fully identified. Although successful embryo development is dependent upon genetic and epigenetic contributions from both the male and female [3], the male potential to adversely affect embryo quality and development is probably underestimated. However, advances in in vitro fertilization (IVF) and embryo transfer (ET) methodologies permit growing insight into the relative male contribution to impaired conceptus development and survival.

2. Male variability

Differences occur among bulls in embryo survival and development in both in vivo and in vitro systems [4]. Earlier work indicated that bulls used for AI differed in embryo mortality rates [5], and that greater losses occurred with low fertility bulls compared with bulls of high fertility [6]. Individual bulls differed in their contribution to embryo development, despite similar fertilization and cleavage rates following IVF [7]. Similarly, rams from two different genetic sources had differing rates of embryonic loss, despite comparable fertilizing capacity [8].

Bulls of differing field fertility (73, 70 and 65%) were not different in embryo cleavage rates, although survival to morulae or beyond favored ($P < 0.10$) the high-fertility group [9]. In contrast, semen from bulls of

* Tel.: +61 2 6933 2652; fax: +61 2 6933 2812.
E-mail address: pchenoweth@csu.edu.au.

0093-691X/$ – see front matter.
doi:10.1016/j.theriogenology.2007.04.002

lower field fertility had reduced in vivo ability to both penetrate oocytes and sustain embryo development than did semen from bulls of higher fertility [10].

In general, any spermatogenic disturbance which results in an elevated incidence of observed abnormal sperm has potential to adversely affect a greater percentage of the sperm population than those detected, at least by conventional means. In such populations, sperm which appear to be morphologically normal may, in fact, be compromised in terms of their ability to either achieve fertilization or maintain a viable pregnancy [11,12].

Thus, in bulls, differences in reproductive success are often not explained by conventional semen assessment. Here, additional clues may be derived by using sophisticated in vitro techniques. For example, bull differences have been reported for initiation and length of the zygotic S-phase, as well as for embryo cleavage and development [9]. In addition, both sperm ability to access the ovum, and accessory sperm numbers, have shown significant individual bull variation [13–15].

3. Semen characteristics

Seminal traits are associated with failure of both fertilization and of embryogenesis [4] with both sperm viability and morphology being linked with early embryonic failure. Much of this male-associated loss is considered to occur relatively early. For example, in dairy cattle, male-associated pregnancy loss is rare after 24 days post-AI [16]. Implicating factors have included elevated ambient and scrotal temperatures, out-of-season rams, immature and aged sperm [17]. In humans abnormal sperm morphology has been associated with repeated spontaneous abortion [18].

Individual boars differ in the insemination dose required to consistently produce the greatest number of offspring [19]. Here, traditional semen assessments tended to be predictive of litter size up to a point, beyond which differences in litter size could not be attributed to observed semen differences. In swine, in vitro oocyte penetration rates were influenced by a number of traits, including sperm morphology, ATP content, motility, acrosome status, hypo-osmotic swelling, percentage of live–dead and osmotic resistance [20]. Several of these assessed sperm characteristics were related to each other. Such results illustrate difficulties in predicting sperm fertility from one or two tests, while reinforcing the need to identify biological markers which reflect unifying mechanisms for many aspects of sperm damage.

4. Sperm oxidative damage

One such ubiquitous underlying mechanism of sperm damage is oxidative damage. Supra-physiological levels of reactive oxygen species (ROS) are considered to play a key role in male infertility [21]. Reactive oxygen species occur in different guises, including those encompassing oxygen free radicals, such as the superoxide anion (O_2), hyperoxyl radical (HOO) and hydroxyl radical (OH) and biologically important non-radical entities including hydrogen peroxide (H_2O_2) and hypochlorous acid (HOCl). Reactive oxygen species are involved in most normal sperm functions including motility activation, capacitation, the acrosome reaction and hyperactivated motility. However, problems occur, particularly with sperm membranes, DNA and midpieces, when imbalances occur. It appears that sperm mitochondrial DNA is more susceptible to oxidative attack than nuclear DNA [22,23]. Factors associated with oxidative stress in male gametes include heat, cigarette smoking, heavy metals, ionizing radiation, gossypol toxicity, zinc deficiency, ageing, cryopreservation and transitional phases in seasonal breeders.

Causes of ROS imbalances in semen include both sperm-mediated and extra-sperm factors. Here, it is noteworthy that hydrogen peroxide can induce DNA fragmentation in human spermatozoa at doses that do not suppress their fertilizing potential [24], and that it has been associated with loss of sperm motility, premature acrosome loss and failure of zona pellucida penetration [25].

In turn, increased DNA damage in the male gamete has been associated with poor semen quality including sperm count, morphology and motility [26], low fertilization rates, impaired pre-implantation development, increased abortion and elevated disease levels in offspring [21,26].

Sperm, as well as critical phases of spermiogenesis, are particularly susceptible to ROS-mediated damage for several reasons, including the following:

- There is a period of increased vulnerability during chromatin condensation.
- Sperm lack DNA repair mechanisms.
- Sperm membranes contain high concentrations of unsaturated fatty acids.
- Sperm themselves generate ROS, particularly during passage through the epididymis [25].
- Sperm possess low levels of cytosolic antioxidant enzymes.
- Sperm spend protracted periods as isolated cells in both the male and female tracts.

- The retention of excess residual cytoplasm (i.e. as retained droplets on the midpiece region) is associated with high levels of ROS generation) [25,27].

This list of sperm vulnerabilities suggest that a significant portion of male-mediated EPL can be explained in terms of the following:

(1) Abnormal sperm head morphology is associated with DNA damage [26].
(2) The major cause of DNA damage in the male gamete is oxidative stress [21,25].
(3) Sperm DNA abnormalities are a major cause of male-factor sub-fertility [21].
(4) Routine sperm assessment parameters are only partially successful in identifying such damage [12].

5. Bio-markers for sperm damage

The spermatogenic epithelium and developed sperm have limited capabilities to respond to stressors, with the result that many different sperm ''problems'' represent the outcomes of a limited number of pathogenic pathways or mechanisms. These, in turn, can reveal themselves via ''bio-markers'' such as the ''diadem/crater defect'', retained cytoplasmic droplets and morphological abnormalities of the sperm acrosome and midpiece.

With common underlying mechanisms at work, it would not be unexpected to find a number of sperm abnormalities occurring consistently, either concurrently or in series. Indeed, in a human study [28], significant positive relationships occurred between levels of sperm ROS production and the proportion of sperm with abnormal head shapes, acrosome abnormalities, midpiece defects, cytoplasmic droplets and tail defects. An example of a consistent pattern of sperm morphological abnormalities occurs with the diadem/crater defect, which represents part of a stereotyped temporal spermatogenic response to a wide variety of stressors, with oxidative damage being a common theme. This response has been characterized in different species using a testicular insulation model as developed for bulls [29], where a consistent temporal series of abnormalities was associated with both duration and severity of stress [30].

Sperm containing diadem/crater defects resulted in lowered embryo quality and survivability [31]; even those with subtle (i.e. non-head-distorting) forms of the diadem/crater defect could gain access to the ovum, leading to both lowered fertility and decreased embryo quality [31,32]. In more recent work with human IVF, micro-injected normal-contour sperm containing vacuoles lowered pregnancy and elevated abortion rates [33].

As well as the diadem/crater defect, a number of ''bio-markers'' for spermatogenic damage may be identified, as shown in the examples below:

(1) Abnormal sperm chromatin
  - morphology (e.g. diadem/crater defect);
  - sperm chromatin structure assay (SCSA).
(2) Abnormal sperm membranes
  - morphology (e.g. loose membranes, retained cytoplasmic droplets);
  - live/dead staining (e.g. nigrosin/eosin);
  - hypo-osmotic swelling test;
  - targeted fluorochromes.
(3) Acrosome loss and/or dysfunction
  - morphology (e.g. percent intact acrosomes, knobbed acrosomes);
  - targeted fluorochromes.
(4) Mitochondrial dysfunction
  - morphology (e.g. abnormal midpiece morphology);
  - targeted fluorochromes.

This indicates that a limited number of procedures, individually or in combination, may be useful in identifying markers for those underlying mechanisms which lead to spermatogenic damage. One such tool is accurate sperm morphology assessment, which can identify the end results of a number of these processes. In turn, qualitative and quantitative assessment of such markers should improve the diagnostic and prognostic value of semen evaluations.

6. Antioxidants

Oxidative stress in sperm is very much influenced by the presence of antioxidants in semen, as reflected in lowered semen antioxidant levels, or activity, in infertile men [27,34]. Despite this, variable results have been obtained with a number of studies on the effects of antioxidants on semen quality in domestic animals. For example, antioxidant intake in healthy non-smoking males did not appear to improve sperm chromatin integrity [35]. On the other hand, human males with elevated levels of DNA-fragmented sperm had these markedly reduced by oral administration of two antioxidants (Vitamins C and E) for 2 months [36]. Similarly, formaldehyde-induced oxidative damage to rat testes was prevented by Vitamin E administration [37]. In bulls, a protective effect of Vitamin E was

reported for gossypol-induced damage to spermatogenesis [38]. With sheep, addition of different antioxidants to extended ram semen generally improved storage characteristics as well as fertilized ova recovery rates [39]. The relative effectiveness of antioxidant therapy is probably associated with factors such as the relative imbalance of reactive oxygen species at the time of administration of the antioxidant in question, and the ability of that antioxidant to counter the particular oxidative threat involved.

7. Chromosome anomalies

Chromosome anomalies, including aneuploidy and Y chromosome deletions, can play a role in male-factor infertility. The male role in numerical chromosomal zygotic anomalies, although difficult to precisely quantify, is undeniable [40]. Structural chromosome abnormalities are linked with approximately 3–6% of spontaneous abortions in humans [40]. Those involving breaks and re-attachment rearrangements are essentially paternal in origin, as are approximately 35% of Robertsonian translocations [40]. In humans, trisomy 21 has been shown to be 20% paternal in origin, and Klinefelter's syndrome 40% [40]. Numerical chromosome abnormalities such as deletion, trisomy and triploidy have been shown to contribute to early embryonic mortality in domestic animals [6]. In infertile men with poor semen quality, a direct relationship has been suggested between the impairment of spermatogenesis (as reflected in morphologically and cytogenetically abnormal germ cells) and rates of baseline aneuploidy in normal spermatozoa [41] with most sperm aneuploidies being associated with lower fertilization rates, as well as reduced embryo survival.

A genetic basis for spermatogenic failure, at least in humans, has been identified for deletions on the Y chromosome, which are associated with infertility especially when azoospermia is involved [42]. Such deletions occur at a relatively high level, indicating that the Y chromosome is susceptible to loss of genetic material [27], not only due to genetic faults but also following exposure to certain environmental agents.

Interesting links have been detected between sperm morphological abnormalities and structural chromosomal aberrations, with the latter being significantly elevated in human sperm with head abnormalities [43]. In turn, animal models have shown that abnormal karyotypes are substantially higher in oocytes injected with severely deformed sperm heads [44].

8. Environmental effects

Elevated temperatures have long been known to result in spermatogenic dysfunction. For example, in mice, acute scrotal heating in males resulted in lowered pregnancy rates and embryo weights in mated females [45], as did both heating and irradiation in male rats [46]. Bull sperm collected following mild thermal testicular insult showed less stable DNA, and more abnormal shapes, than sperm obtained pre heat stress [47]. Individual bulls have been shown to vary in their spermatogenic response to scrotal insulation [48]. Chronological age has been also associated with increased chromosomal abnormalities in human sperm, which can occur in the absence of observed sperm morphological abnormalities [49].

Seasonal effects on EED occur with sheep, where semen collected from rams collected during ''long'' days induced higher rates of embryonic mortality (measured as the difference between 18 day pregnancy and lambing rates) than that from rams collected during ''short'' days [50].

In lactating dairy cows, increased embryonic loss has been associated with ET (compared with AI), high milk production, and elevated rectal temperatures [51]. In addition, the cryopreservation process may play a role in inducing sperm DNA damage, particularly in human subjects already categorized as infertile [21]. In cattle, a general assumption is that freeze–thawing of sperm leads to a 50% reduction of sperm viability and an 85% reduction in fertilizing ability.

9. Temporal relationships

Several different temporal relationships are associated with embryo ''success'', although these are not necessarily directly associated with the male.

Firstly, time of insemination in relation to estrus or ovulation is important for success of both fertilization and embryo quality in cattle, although these differ in time constraints. For example, in cattle, early insemination resulted in poor fertilization rates (i.e. low numbers of accessory sperm) but good embryo quality, whereas late insemination resulted in high fertilization rates (high numbers of accessory sperm) but poor embryo quality [4].

Time of semen storage also influences embryonic viability. With extended ram semen, time of storage (0–3 days), was associated with increasing rates of embryonic loss, as well as lowered fertility [52]. This confirmed earlier work with dairy cattle which indicated that storage time of extended semen resulted in

decreased fertility and increased embryonic loss (calculated as the difference between 1 and 5 months non-return rate) [53]. However, such an effect was not evident with frozen porcine semen [54] or with frozen bull semen [55].

Time of sperm/oocyte association can influence results. For example, a shorter (1 h) ''exposure'' of human oocytes to spermatozoa improved fertilization rate and embryo quality compared with longer ($\geq$16 h) when used with male-factor infertility cases [56].Lastly, ageing of the male semen donor does have interesting effects on embryo development. Here, sperm from older males represent the product of more spermatogonial germ cell divisions than those from younger males. This, in turn, provides more opportunity for replication errors. Thus, the amount of DNA damage in human sperm in males aged 36–57 years was three times higher than that of males aged 36–57 years, despite observed differences in fertility, sperm count and other measures of semen quality [57].

10. Infectious agents

Although semen is an important vector for viral diseases, testing for viruses in semen has not been widespread. This is despite growing evidence that the virus–sperm interaction can have a number of adverse consequences. Viruses have been found in both testicular compartments and they can be protected by the blood–testis barrier from body defense mechanisms and treatments, allowing the testis to become a viral reservoir. Vertical transmission of viruses via the germ line is well established. In this discussion, interest is primarily focused on the possible effects of viruses on fertilization and embryo development.

In humans, HIV virus has been shown to not only attach to spermatozoa, but also to enter the cell through intact plasma membranes, although it is still unclear whether or not the virus can replicate within the cell. However, both seminal mononuclear cells and sperm were found to harbor pro-viral sequences. HIV-particles not only bind to and enter sperm, but such sperm can transfer HIV-1 like particles to human oocytes [58]. In monkeys, SIV causes hypo-spermatogenesis in conjunction with degeneration of the seminiferous epithelium. In cats, FIV is shed in semen and has been associated with sperm abnormalities. It also affects the hypothalamic–pituitary–gonadal axis, such that affected males have lowered testosterone production.

Cytomegalovirus (CMV; a member of the Herpesviridae) is ubiquitous in the human population, and although infection is lifelong, it is usually latent. However, it may be activated by another infection, or by lowered immunocompetence. In the US, CMV is probably the most important agent responsible for congenital infection and damage to embryos in humans [59]. In mice, CMV is believed to be harbored in the testes and to replicate in germ cells; it has been recovered from both epididymal sperm and from the seminal vesicles. Cytomegalovirus causes necrotic tissue changes in the testicles of rhesus monkeys.

Another herpesvirus, HSV, is associated with fertility problems in man and has been detected within spermatozoa by in situ hybridization. Equine herpesvirus 1 (EHV-1) has been shown to replicate in the testes and be shed in semen [60]; although it evidently does not replicate in the seminiferous epithelium, it does cause increased abnormalities, particularly of the sperm head and midpiece [60]. Bovine herpesvirus 1 (BHV-1) is prevalent in cattle populations, is transmitted in semen, causes infertility and can recrudesce in bulls in response to stress or lowered imunocompetence. It can be excreted in semen without a serum antibody response [61].

Adenovirus has also been detected in semen of infertile men, and has been used as a vector to transfer foreign DNA into the sperm nucleus of pigs and from there, into offspring. In swine, PRRS virus replicates in testicular germ cells, is transmitted in semen, causes spermatogenic dysfunction, and has been detected in spermatogenic cells as well as in macrophages [62,63]. In addition, rubalavirus can cause severe epididymo-orchitis and reduced semen quality in sexually mature boars [64]. Human papillomavirus (HPV) DNA has been detected within human sperm, and appears to adversely affect semen quality as well as be capable of infecting the uterus and embryo [65,66].

The detection of a number of viruses in bull semen is a source of ongoing concern for the AI industry. Bovine viral diarrhea virus (BVDV) virus can occur at high concentrations in semen, and is transmitted in semen, although it has not been associated with overt sperm defects. Conversely, Bluetongue virus (BTV, an orbivirus), also transmitted by bull semen [67], has been associated with sperm abnormalities with virus-like particles being detected in the sperm nuclei of affected individuals [68]. Bluetongue virus has been associated with a number of reproductive disorders including EED, abortion, teratogenic defects in both calves and lambs as well as transient infertility in bulls and rams [69].

Although not a virus, *Ureaplasma urealyticum* can cause embryo loss without necessarily affecting apparent sperm quality. In one study, sperm from infected human males had a low percent stable chromatin with a high

percent denatured DNA; both improved after antibiotic treatment. Sperm infected in vitro showed significant dose and time-dependent chromatin decondensation and DNA damage. Such damage was associated with impaired embryo development despite a high fertilization rate [70]. In naturally mated beef cattle, epidemiological evidence supported the possibility of venereal transmission of *U. diversum* [71]. In vitro work with *Mycoplasma bovis* showed that it can adversely affect fertilization [72]. More recently, *M. hominis* has been shown to both attach to, and invade, human sperm with no immediate effects on sperm or morphology viability [73]; this supports the observation that *U. diversum* can attach strongly to bovine sperm heads and midpieces (Chenoweth and Brown, unpublished) and was consistent with the observation that *U. diversum* (as well as mycoplasmas) could not be removed from bovine embryos by washing after artificial exposure [74]. It is possible that both ureaplasmas and mycoplasmas can induce DNA fragmentation (via decondensation, denaturation or single DNA strand breaks) which do not result in immediate, obvious sperm damage, but lead to post-fertilization problems with embryo development and implantation [70,73].

In summary, there appears to be increasing evidence, gleaned particularly from ET and IVF studies, for adverse male effects on fertility that occur subsequent to fertilization. These effects may be expressed in terms of impaired embryo viability, implantation and development and possibly also in compromised fetal survival. Links have been established between early pregnancy failure and a number of male-factor traits, including sperm abnormalities, chromatin and chromosomal anomalies and infectious agents. However, in many cases, the detected abnormalities represent a symptom of wider underlying damage. As spermatogenesis has a restricted repertoire of responses available in the face of a variety of stressors, a number of male-factor traits are linked via common pathogenic mechanisms which, in turn, produce their own biomarkers. In turn, these biomarkers provide opportunities to identify and quantify spermatogenic damage, and, perhaps, to offer preventive or remedial measures.

References

[1] Betts DH, King WA. Genetic regulation of embryo death and senescence. Theriogenology 2001;51:171–91.

[2] Grimard B, Freret S, Chevallier A, Pinto A, Ponsart C, Humblot P. Genetic and environmental factors influencing first service conception rate and late embryonic/foetal mortality in low fertility dairy herds. Anim Reprod Sci 2006;91: 31–44.

[3] Duranthon V, Renard JP. The developmental competence of mammalian oocytes: a convenient but biologically fuzzy concept. Theriogenology 2001;55:1277–89.

[4] Saacke RG, Dalton JC, Nadir S, Nebel RL, Bame JH. Relationship of seminal traits and insemination time to fertilization rate and embryo quality. Anim Reprod Sci 2000;60–61:663–77 [special issue].

[5] Bulman DC. A possible influence of the bull on the incidence of embryonic mortality in cattle. Vet Rec 1979;105:420–2.

[6] Courot M, Colas G. The role of the male in embryonic mortality. In: Greenan JM, Diskin MG, editors. Embryonic mortality in farm animals. Dordrecht: Martinus Nijhoff; 1986. p. 196–206.

[7] Eyestone WH, First NL. Variation in bovine embryo development in vitro due to bulls. Theriogenology 1989;31:191 [abstract].

[8] Maxwell WMC, Quintana-Casares PI, Setchell BP. Ovulation rate, fertility, and embryo mortality in ewes mated to rams from two different strains. Proc Aust Soc Anim Prod 1992;19:192–4.

[9] Schneider CS, Ellington JE, Wright Jr RW. Effects of bulls with different field fertility on in vitro embryo cleavage and development using sperm co-culture systems. Theriogenology 1999;51:262 [abstract].

[10] Hillery FL, Parrish JJ, First NL. Bull specific effect on fertilization and embryo development in vitro. Theriogenology 1990;33:249 [abstract].

[11] Smorag Z, Bocheneck M, Wojdan Z, Sloniewski K, Reklewski Z. The effect of sperm chromatin structure on quality of embryos derived from superovulated heifers. Theriogenology 2000;53: 201 [abstract].

[12] Ballachey BE, Evanson DP, Saacke RG. The sperm chromatin structure assay: relationship with alternate tests of semen quality and heterospermic performance of bulls. J Androl 1988;9:109–15.

[13] De Jarnette JM, Saacke RG, Bame J, Vogler CJ. Accessory sperm: their importance to fertility and embryo quality, and attempts to alter their numbers in artificially inseminated cattle. J Anim Sci 1992;70:484–91.

[14] Nadir S, Saacke RG, Bame J, Mullins J, Degelos S. Effect of freezing semen and dosage of sperm on number of accessory sperm, fertility and embryo quality in artificially inseminated cattle. J Anim Sci 1993;71:199–204.

[15] den Daas JHG, DeJong G, Lansbergen L, Van Wagtendonk- De Leeuw AM. The relationship between the number of spermatozoa inseminated and the reproductive efficiency of individual bulls. J Dairy Sci 1998;81:1714–23.

[16] Humblot P. Relative incidence of late embryonic mortality and post-insemination anoestrus in late returns to oestrus in dairy cows. Curr Top Vet Med Anim Sci 1982;20:298–304.

[17] Saacke RG, Nadir S, Nebel RL. Relationship of semen quality to sperm transport, fertilization and embryo quality in ruminants. Theriogenology 1994;41:45–50.

[18] Hill JA, Abbott AF, Politch JA. Sperm morphology and recurrent abortion. Fert Steril 1994;61:776–8.

[19] Flowers WL. Increasing fertilization rate of boars: Influence of number and quality of spermatozoa inseminated. J Anim Sci 2002;80(E.Suppl. 1):E47–53.

[20] Gadea J, Matas C. Sperm factors related to in vitro penetration of porcine oocytes. Theriogenology 2000;54:1343–57.

[21] Lewis SEM, Aitken RJ. DNA damage to spermatozoa has impacts on fertilization and pregnancy. Cell Tissue Res 2005;322: 33–41.

[22] Sawyer DE, Roman SD, Aitken RJ. Relative susceptibilities of mitochondrial and nuclear DNA to damage induced by hydrogen

peroxide in two mouse germ cell lines. Redox Rep 2001;6: 182–94.
[23] Bennetts LE, Aitken RJ. A comparative study of oxidative DNA damage in mammalian spermatozoa. Mol Reprod Dev 2005;71: 77–87.
[24] Aitken RJ, Gordon E, Harkiss D, Twigg J, Milne P, Jennings Z, et al. Relative impact of oxidative stress on the functional competence and genomic integrity of human spermatozoa. Biol Reprod 1998;59:1037–46.
[25] Aitken RJ. Active oxygen in spermatozoa during epididymal transit. In: Robiare B, Hinton B, editors. The epididymis. Kluwer Academic/Plenum Publishers; 2002.
[26] Erenpreiss J, Spano M, Erenpreisa J, Bungum M, Giwercman A. Sperm chromatin structure and male fertility: biological and clinical aspects. Asian J Androl 2006;8:11–29.
[27] Aitken RJ, Sawyer. The human spermatozoon—not waving but drowning. In: Robaire B, Hales BF, editors. Advances in male mediated developmental toxicity. Kluwer Academic/Plenum Publishers; 2003. p. 85–98.
[28] Said TM, Aziz N, Sharma K, Lewis-Jones I, Thomas AJ, Agarwai A. Novel association between sperm deformity index and oxidative stress-induced DNA damage in infertile male patients. Asian J Androl 2005;7:121–6.
[29] Vogler CJ, Bame JH, DeJarnette JM, McGilliard ML, Saacke RG. Effects of elevated testicular temperature on morphology characteristics of ejaculated spermatozoa in the bovine. Theriogenology 1993;40:207–19.
[30] Chenoweth PJ. Genetic sperm defects. Theriogenology 2005;64: 457–68.
[31] Saacke RG, DeJarnette JM, Bame JH, Karabinus DS, Whitman S. Can spermatozoa with abnormal heads gain access to the ovum in artificially inseminated super- and single-ovulating cattle? Theriogenology 1998;51:117–28.
[32] Miller D, Hrudka M, Cates WF, Mapletoft R. Infertility in bulls with a nuclear sperm defect. Theriogenology 1982;17:611–21.
[33] Berkovitz A, Eltes F, Ellenbogen A, Peer S, Feldberg D, Bartoov B. Does the presence of nuclear vacuoles in human sperm selected for ICSI affect pregnancy outcome? Hum Reprod 2006;21:1787–90.
[34] Koca Y, Ozdal OL, Celik M, Unal S, Balaban N. Antioxidant activity of seminal plasma in fertile and infertile men. Arch Androl 2003;49:355–9.
[35] Silver EW, Eskenazi B, Evenson DP, Block G, Young S, Wyrobek AJ. Effect of antioxidant intake on sperm chromatin stability in healthy non-smoking men. J Androl 2005;26:550–6.
[36] Greco E, Iacobelli M, Rienzi L, Ubaldi F, Ferrero S, Tesarik J. Reduction of the incidence of sperm DNA fragmentation by oral antioxidant treatment. J Androl 2005;26:349–53.
[37] Diang-Xia Z, Shu-Dong Q, Jie Zhang, Hong T, Hai-Xue W. The protective effect of vitamin E against oxidative damage caused by formaldehyde in the testes of adult rats. Asian J Androl 2006;8:584–8.
[38] Velasquez-Pereira J, Chenoweth PJ, McDowell LR, Risco CA, Staples CR, Prichard D, et al. Reproductive effects of feeding gossypol and vitamin E in bulls. J Anim Sci 1998;76: 2894–904.
[39] Maxwell WMC, Stojanov T. Liquid storage of ram semen in the absence or presence of some antioxidants. Reprod Fert Dev 1996;8:1013–20.
[40] Mattei JF. Influence of the male in embryonic mortality. In: Courot M, editor. The male in farm animal reproduction. Boston: Martinus Nijhoff; 1984. p. 350–69.
[41] Bernadini L, Borini A, Preti S, Conte N, Flamigni C, Capitanio GL, et al. Study of aneuploidy in normal and abnormal germ cells of fertile and infertile men. Hum Reprod 1998;13:3406–13.
[42] Krausz C, Quintana-Murci L, Barbaux S, Siffroi J-P, Rouba H, Delafontaine D, et al. A high frequency of Y chromosome deletions in males with nonidiopathic infertility. J Clin Endocr Metab 1999;84:3606–12.
[43] Lee JD, Kamigichi Y, Yanagimachi R. Analysis of chromosome constitution of human spermatozoa with normal and aberrant head morphologies after injection into mouse oocytes. Hum Reprod 1996;11:1942–6.
[44] Kishikawa H, Tateno H, Yanagimachi R. Chromosome analysis of BALB/c mouse spermatozoa with normal and abnormal head morphology. Biol Reprod 1999;61:809–12.
[45] Jannes P, Spiessens C, Van der Auwera I, D'Hooghe T, Verhoven G, Vanderschueren D. Male, subfertility induced by acute scrotal heating affects embryo quality in normal female mice. Hum Reprod 1998;13:372–5.
[46] Setchell BP, D'Occhio MJ, Hall MJ, Laurie MS, Tucker MJ, Zupp JL. Is embryonic mortality increased in normal female rates mated to subfertile males? J Reprod Fert 1988;82:567–74.
[47] Karabinus D, Vogler CJ, Saacke RG, Evenson DP. Chromatin structural changes in bovine sperm after scrotal insulation of Holstein bulls. J Androl 1997;18:549–55.
[48] Walters AH, Eyestone WE, Saacke RG, Pearson RE, Gwazdauskas FC. Bovine embryo development after IVF with spermatozoa having abnormal morphology. Theriogenology 2005;63:1925–37.
[49] Rosenbusch B, Strehler E, Sterzik K. Cytogenetics of human spermatozoa: correlations with sperm morphology and age of fertile men. Fert Steril 1992;58:1071–3.
[50] Colas G. Factors affecting the quality of ram semen. In: Haresign W, editor. Sheep production. London: Butterworths; 1983. p. 453–65.
[51] Vasconcelos JLM, Demetrios DGB, Santos RM, Chiari JR, Rodriguez CA, Sa Filho OG. Factors potentially affecting fertility of lactating dairy cow recipients. Theriogenology 2006;65:192–200.
[52] Salamon S, Maxwell WMC. Storage of ram semen. Anim Reprod Sci 2006;2:77–111.
[53] Salisbury GW, Bratton RW, Foote RH. The effect of time and other factors on the non-return to service estimate of fertility level in artificial insemination of cattle. J Dairy Sci 1952;35: 256–60.
[54] Stroble KA, Stewart TS, Krisher RL. Duration of crypreservation has no effect on fertilizing ability of boar spermatozoa. Theriogenology 2003;59:212.
[55] Hallap T, Nagy S, Haard M, Jaakma U, Johannisson A, Rodriguez-Martinez H. Sperm chromatin stability in frozen-thawed semen is maintained over age in AI bulls. Theriogenology 2005;63:1752–63.
[56] Gianaroli L, Fiorentino A, Magli C, Ferraretti A, Montanaro N. Prolonged sperm–oocyte exposure and high sperm concentration affect human embryo viability and pregnancy rate. Hum Reprod 1996;11:2507–11.
[57] Aitken RJ, Koopman P, Lewis SEM. Seeds of concern. Nature 2004;432:48–52.
[58] Bacetti B, Benedetto A, Burni AG, Collodel G, Ceccarini EC, Crisa N, et al. HIV-Particles in spermatozoa of patients with AIDS and their transfer into the oocyte. J Cell Biol 1994;127: 903–14.
[59] Hammitt DG, Aschenbrenner DW, Williamson RA. Culture of cytomegalovirus from frozen-thawed semen. Fert Steril 1988;49: 554–7.

[60] Tearle JP, Smith KC, Boyle MS, Binns MM, Livesay GJ, Mumford JA. Replication of equid herpesvirus (EHV-1) in the testes and epididymides of ponies and venereal shedding of infectious virus. J Comp Pathol 1996;115:385–97.
[61] Eaglesome MD, Garcia MM. Disease risks to animal health from artificial insemination with bovine semen. Rev Sci Tech Off Int Epiz (OIE) 1997;16:215–25.
[62] Sur J-H, Doster AR, Christian JS, Galeota JA, Wills RW, Zimmerman JF, et al. Porcine reproductive and respiratory syndrome virus replicates in testicular germ cells, alters spermatogenesis and induces germ cell death by apoptosis. J Virol 1997;71:9170–9.
[63] Christopher-Hennings J, Nelson EA, Nelson JK, Rossow KD, Shivers JL, Yaeger MJ, et al. Identification of porcine reproductive and respiratory virus in semen and tissues from vasectomised and nonvasectomised boars. Vet Pathol 1998;35:260–7.
[64] Ramirez-Mendoza H, Hernandez-Jauregui P, Reyes-Leyva J, Zenteno E, Moreno-Lopez J, Kennedy S. Lesions in the reproductive tract of boars experimentally infected with porcine rubalavirus. J Comp Pathol 1997;117:237–52.
[65] Chan PJ, Seraj IM, Kalugdan TH, King A. Evidence for ease of transmission of human papilloma virus DNA from sperm to cells of the uterus and embryo. J Appl Reprod Genet 1996;13:516–9.
[66] Henneberg AA, Patton WC, Jacobson JD, Chan PJ. Human papilloma virus DNA exposure and embryo survival is stage-specific. J Assist Reprod Genet 2006;23:255–9.
[67] Howard TH, Bowen RA, Pickett BW. Isolation of bluetongue virus from bull semen. In: Bluetongue and related orbiviruses. Alan R. Liss, Inc.; 1985. p. 127–34.
[68] Foster NM, Alders MA, Luedke AJ, Walton TE. Abnormalities and virus-like particles in spermatozoa from bulls latently infected with bluetongue virus. Am J Vet Res 1980;41:101–8.
[69] Osburn B. The impact of bluetongue virus on reproduction. Comp Immunol Microbiol Infect Dis 1994;17:189–96.
[70] Reichart M, Kathane I, Bartoov B. In vivo and in vitro impairment of human and ram nuclear chromatin by sexually transmitted *Ureaplasma urealyticum* infection. Biol Reprod 2000;63:1041–8.
[71] Rae DO, Chenoweth PJ, Brown MB, Genho SA, Moore SA, Jacobsen KE. Reproductive performance of beef heifers: effects of vulvo-vaginitis, Ureaplasma diversum and prebeeding antibiotic administration. Theriogenology 1993;40:497–508.
[72] Eaglesome MD, Garcia MM. The effect of *Mycoplasma bovis* on fertilization processes in vitro with bull spermatozoa and zona-free hamster oocytes. Vet Microbiol 1990;21:329–37.
[73] Díaz-García FJ, Herrera-Mendoza AP, Giono-Cerezo S, Guerra-Infante FM. *Mycoplasma hominis* attaches to and locates intracellularly in human spermatozoa. Hum Reprod 2006;21:1591–8.
[74] Stringfellow DA, Givens MD. Infectious agents in bovine embryo production: hazards and solutions. Theriogenology 2000;53:85–94.