Rev. Bras. Reprod. Anim., Belo Horizonte, v.35, n.2, p.154-159, abr./jun. 2011. Disponível em www.cbra.org.br

Effects of the male on the embryo

Efeitos do macho sobre o embrião

P.J. Chenoweth

Charles Sturt University, EH Graham Centre for Agricultural Innovation, Wagga Wagga, NSW 2678, Australia.
Corresponding author: pchenoweth@csu.edu.au

Abstract

Early pregnancy failure or loss (EPL) represents a major source of wastage and inefficiency in livestock production systems, with increasing evidence that male factors can play a significant role in this loss. Of those adverse male effects which have been identified, those associated with sperm damage, particularly sperm DNA, have been best characterized. Here the case is made that there are a limited number of mechanisms by which the spermatogenic epithelium can respond to stressors, leading to a predictable trail of detectable biomarkers. Potential stressors include pathogens, especially viruses, which can directly damage sperm as well as be transmitted in semen and/or sperm with important implications for both natural and artificial breeding.

Keywords: early pregnancy loss, male infertility, sperm damage.

Resumo

A falta ou perda precoce da gestação representa uma grande fonte de perda e ineficiência em sistemas de produção bovina, com crescente evidência de que fatores ligados ao macho podem ter um papel significativo nestas perdas. Dos efeitos adversos que foram identificados, os associados com danos do esperma, particularmente o seu DNA, foram mais bem caracterizados. Aqui se defende que há um número limitado de mecanismos pelos quais a membrana do espermatozoide pode responder a desafios deletérios, levando a uma trilha previsível de biomarcadores detectáveis. Desafios potenciais incluem elementos patogênicos, especialmente vírus, que podem danificar o espermatozoide diretamente, além de serem transmitidos no sêmen com implicações importantes para a reprodução natural e artificial.

Palavras-chave: *perda precoce da gravidez, infertilidade do macho, dano e esperma.*

Introduction

Male effects in reproduction can be obvious or discreet and both positive and negative. They include not only direct effects on fertilization, but also indirect effects on the quality and viability of the conceptus.

Male associated differences

Embryo development relies not only upon maternal properties but also on paternal factors (Duranthon and Renard, 2001). For example, differences have been shown to occur among bulls in embryo survival and development both in-vivo and in-vitro (Saacke et al., 2000). Earlier work showed that AI bulls of low fertility had higher rates of embryonic loss than did bulls of high fertility (Courot and Colas, 1986). Similarly with sheep, Maxwell et al. (1992) reported that rams of differing genetic lines differed in rates of embryonic loss despite comparable fertilizing capacity. Differences in bull reproductive success are often not explained by conventional seminal examination. Here, advances in in-vitro fertilization (IVF) and embryo transfer (ET) methodology have improved capabilities to detect differences in male effects on both fertilization and embryo development. Using such techniques, bull differences have been reported for IVF rates, initiation and length of the zygotic S-phase, and in embryo cleavage and development (Schneider et al., 1999). Other findings include great individual variation in sperm in-vivo ability to access the ovum as well as in accessory sperm numbers (Nadir et al., 1993).

Using frozen semen from bulls of differing field fertility (73, 70 and 65%), Schneider et al. (1999) obtained no difference in embryo cleavage rates, although survival to morulae or beyond favored the higher fertility group ($P < 0.10$). In contrast, Hillery et al. (1990) observed that semen from bulls of lower field fertility showed reduced in-vivo ability to both penetrate oocytes and sustain embryo development than semen from bulls of higher fertility.

It should be noted that a disturbance in spermatogenesis which results in an elevated incidence of observed abnormal sperm, most probably adversely affects a greater percentage of the sperm population than those detected (Vogler et al., 1993), with such damage including defective chromatin (Ballachey et al., 1988; Avenado and Oehninger, 2010).

Chromosome anomalies

Chromosome anomalies, including aneuploidy and Y chromosome deletions, can contribute to male-factor infertility. The male role in numerical chromosomal zygotic anomalies, although difficult to precisely quantify, is undeniable (Bernadini et al., 1998). Structural chromosome abnormalities are involved in approximately 3-6% of human spontaneous abortions. Those involving breaks and re-attachment rearrangements are essentially paternal in origin, as are approximately 35% of Robertsonian translocations (Bernadini et al., 1998) which can create problems in cattle populations. In humans, trisomy 21 has been shown to be 20% paternal in origin, and Klinefelters syndrome 40% (Bernadini et al., 1998). Numerical chromosome abnormalities such as deletion, trisomy and triploidy can lead to embryonic mortality in domestic animals (Courot and Colas, 1986). In infertile men with poor semen quality, a direct relationship has been suggested between the impairment of spermatogenesis (as reflected in abnormal germ cells) and aneuploidy (Bernadini et al., 1998); in turn, sperm aneuploidies are linked with lowered 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 have been associated with infertility especially involving azoospermia (Krausz et al., 1999). Such deletions occur at a relatively high level, indicating that the Y chromosome is susceptible to loss of genetic material, not only due to genetic faults but also following exposure to certain environmental agents.

Interesting links have been detected between sperm and chromosome abnormalities and chromosomal, with the latter being significantly elevated in human sperm with head abnormalities (Rosenbusch et al., 1992; Lee et al., 1996).

Semen characteristics

High and variable rates of embryo loss occur in mammals (Betts and King, 2001), with less than half of inseminated human and bovine oocytes reach the blastocyst stage (Betts and King, 2001). Of those that do reach blastocyst stage, many do not implant or attach following ET. Explanations for this high failure rate are not always clear, with much of the earlier work being focused on female factors.

Despite this, the male has long been suspected as a culprit in early pregnancy loss and abortion with both sperm viability and morphology being associated with early embryonic failure (Saacke et al., 2000). Implicating factors have included elevated ambient and scrotal temperatures, “out-of-season” breeding, immature and aged sperm. In bulls, particular sperm abnormalities linked with compromised embryos include diadem defects, proximal droplets and decapitated sperm (Saacke et al., 2000). In pigs, those sperm characteristics related to in-vitro penetration of oocytes included all conventional semen parameters except sperm concentration and eosin-nigrosin (live-dead) staining (Gadea and Matas, 2000), most of which were correlated with each other. Flowers (2002) reported that individuals differed in the insemination dose required to consistently produce the greatest number of pigs. Traditional semen assessments tended to be predictive of litter size up to a point. Beyond this, differences in litter size could not be attributed to observed semen differences. 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.

Sperm oxidative damage

One such common mechanism is oxidative damage, in which supra-physiological levels of reactive oxygen species (ROS) are considered to play a key role in male infertility (Lewis and Aitken, 2005). Reactive oxygen species occur in different guises, including those encompassing oxygen free radicals, such as the superoxide anion, hyperoxyl radical and hydroxyl radical and biologically important non-radical entities including hydrogen peroxide and hypochlorous acid. 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 (Aitken et al., 1998); and that it is associated with loss of sperm motility, premature acrosome loss and failure of zona penetration (Aitken, 2002). Further, it appears that sperm mitochondrial DNA is more susceptible to oxidative attack than is nuclear DNA (Sawyer et al., 2001; Bennets and Aitken, 2005).

In turn, increased DNA damage is linked with poor semen quality including sperm count, morphology and motility (Erenpreiss et al., 2006)), low fertilization rates, impaired pre-implantation development, increased abortion and elevated disease levels (including cancer) in offspring (Lewis and Aitken, 2005) as well as potential infertility in offspring (Erenpreiss et al., 2006). 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.

At this stage it should be emphasized that ROS are also advantageous to a number of sperm functions including motility activation, capacitation, the acrosome reaction and hyperactivated motility. However, sperm

problems occur when imbalances occur, with these being particularly evident in the membranes, DNA and midpieces. When imbalances do occur, sperm, as well as critical phases of spermiogenesis, are particularly susceptible to ROS-mediated damage for several reasons, including;

- increased susceptibility during chromatin condensation (and associated protamine –histone changes),
- lack DNA repair mechanisms
- high concentrations of unsaturated fatty-acids in membranes
- ability to generate ROS, particularly in the epididymis (Aitken, 2002).
- low levels of cytosolic antioxidant enzymes.
- protracted periods as isolated cells in both the male and female tracts.
- retention of excess residual cytoplasm (i.e. as retained droplets on the middlepiece region) which is associated with high levels of ROS generation; Aitken and Krausz, 2001; Aitkin, 2002).

This susceptibility of sperm to oxidative stress is reflected in damage to a variety of ways including damage to nuclear DNA integrity as well as to the structure and function of both mitochondria and membranes (Aitken and Krausz, 2001).

As there is a common underlying mechanism at work, and as both spermatogenesis and sperm themselves have a limited repertoire in which they respond to stressors, it is not unexpected to find a number of such sperm abnormalities occurring either concurrently or in series. For example, the diadem/crater defect of sperm represents part of a stereotyped temporal spermatogenic response to a wide variety of stressors. This response has been characterized in different species using a testicular insulation model where a consistent temporal series of abnormalities is associated with both the duration and severity of stress (Chenoweth, 2005).

Sperm containing diadem/crater defects result in lowered embryo quality and survivability (Saacke et al., 1992); even those with subtle (i.e. non head-distorting) forms of the diadem/crater defect can gain access to the ovum, leading to both lowered fertility and decreased embryo quality (Miller et al., 1982; Saacke et al., 1992; Walters et al., 2005). More recent work with human IVF, using micro-injected sperm containing vacuoles (although of normal contour) showed that they resulted in lowered pregnancy and higher abortion rates (Berkovitz et al., 2006).

Other sperm "bio-markers" include retained cytoplasmic droplets, acrosome abnormalities, midpiece (mitochondrial) aberrations and "tag" proteins such as ubiquitin.

It should be noted that a disturbance in spermatogenesis which results in an elevated incidence of observed abnormal sperm, most probably also has unobserved adverse affects on a greater percentage of the sperm population than those obviously abnormal (Thundathil et al., 2000). Sperm which appear to be morphologically normal may have defective chromatin and lowered fertility (Gledhill, 1966; Evenson et al., 1980). In cattle, higher levels of sperm chromatin damage were associated with higher rates of unfertilized and morphologically abnormal embryos from superovulated heifers (Smorag et al., 2000).

These findings support the following concepts in explaining a significant part of male-mediated EPL as follows:

1. Abnormal sperm head morphology is associated with DNA damage (Erenpreiss, 2006).
2. The major cause of DNA damage in the male gamete is oxidative stress (Aitken, 2002; Lewis and Aitken, 2005).
3. Sperm DNA abnormalities are a major cause of male-factor sub-fertility.
4. Routine sperm assessment parameters are only partially successful in identifying such damage.

Environmental effects

Elevated temperatures have long been known to cause spermatogenic dysfunction. In male mice, acute scrotal heating resulted in lowered pregnancy rates and embryo weights in mated females (Jannes et al., 1998); an effect which also occurs with irradiated males (Setchell et al., 1988). Vogler et al. (1993) showed that 48 hr scrotal insulation in bulls resulted in a stereotyped pattern of sperm abnormalities, with diadem/crater sperm defects predominating by approximately 3 weeks following treatment. In turn, mild thermal insult of bull testes resulted in sperm of lowered DNA stability (Karabinus et al., 1997); a factor associated with reduced sperm performance in heterospermic trials (Ballachey et al., 1988). Age of the male has been also associated with increased chromosomal abnormalities in human sperm (Rosenbusch et al., 1992). Seasonal effects were also suggested in sheep, where semen collected from rams collected during "long" days induced higher rates of embryonic mortality (measured as the difference between 18d pregnancy and lambing rates) than that from rams collected during "short" days (Colas, 1983).

Time of semen storage also influences embryonic loss rates, with storage of extended, chilled ram semen for 0-3 days resulting in time-related increased embryonic loss and reduced fertility (Salamon and Maxwell 2006), confirming results from an earlier study with dairy cattle (Salisbury et al., 1952). However, this does not appear to be the case for cryopreserved semen (Stroble et al., 2003).

Role of infectious agents

Although semen is an important vector for viral diseases, and virus may be detected in all seminal components, testing for viruses in semen has not been widespread despite the impetus provided by research in humans on HIV (Piomboni and Baccetti, 2000) which provides growing evidence of the adverse consequences of viral-sperm interactions. Viruses are found in both testicular compartments (interstitium and seminiferous tubules) and are preserved in frozen semen (Hammitt et al., 1988). They can be shielded from body defense mechanisms and treatments by the blood-testis barrier, allowing the testis to become a viral reservoir. In this discussion, interest is primarily focused on the possible effects of viruses on fertilization and embryo development.

A number of viruses have been detected in bull semen, with important implications for the A.I. industry. BVD virus can occur at high levels in semen, where it is capable of transmission, although it not necessarily causing sperm defects. Conversely, blue-tongue virus (BTV), also isolated from, and transmitted by, bull semen (Howard et al., 1985), has been associated with sperm abnormalities. In addition, virus-like particles have been detected in the sperm nuclei of affected individuals (Foster et al., 1980). BTV is associated with a number of reproductive disorders including EED, abortion, teratological defects (both calves and lambs) and transient infertility in bulls and rams (Osburn, 1994). Foot-and- mouth disease virus (FMDV) can be present in bull semen and transmitted via AI (Cottral et al., 1968). Bovine herpesvirus 1 (BHV-1) is prevalent in cattle populations where it can be transmitted in semen, cause infertility and can recrudesce in bulls following stress or lowered imunocompetence. The papillomavirus can be present in human sperm where HPV-specific genes are transcribed (Lai et al., 1997). Here it appears to adversely affect semen quality as well as be capable of infecting the uterus and embryo (Chan et al., 1996); findings which have important implications for livestock.

Many viruses can be present in boar semen, especially during the viremic phases of infection (Guerin and Pozzi, 2005). Porcine reproductive and respiratory syndrome virus (PRRSV) replicates in testicular germ cells, is transmitted in semen, causes spermatogenic dysfunction, and has been detected in spermatogenic cells as well as in macrophages (Sur et al., 1997; Christopher-Hennings et al., 1998). In addition, rubalavirus can cause severe epididymo-orchitis and reduced semen quality in sexually mature boars (Ramirez-Mendoza et al., 1997).

Although not a virus, *Ureaplasma urealyticum* in humans causes embryo loss without necessarily affecting apparent sperm quality. In one study, in-vitro infected sperm showed significant dose and time-dependent DNA damage (Reichart et al., 2000). In working with *U diversum* in cattle, Rae et al. (1993) detected organisms attached to sperm structures within the plasma membrane; an observation reinforced by more recent work showing that Mycoplasmataceae can attach to sperm intracellularly (Diaz-Garcia et al., 2006).

In conclusion, it would appear that infective agents, particularly viruses, are underestimated as causes of male factor infertility and worthy of greater research focus, particularly in light of their ability to migrate via the sperm into the egg and adversely influence conceptus development and viability.

References

Aitken RJ. Active oxygen in spermatozoa during epididymal transit. In: Robiare B, Hinton B (Ed.).*The epididymis*. New York: Kluwer Academic/Plenum Publishers, 2002. p.435-447.

Aitken RJ, Gordon E, Harkiss D, Twigg J, Milne P, Jennings Z, Irvine DS. Relative impact of oxidative stress on the functional competence and genomic integrity of human spermatozoa. *Biol Reprod*, v.59, p.1037-1046, 1998.

Aitken RJ, Krause C. Oxidative stress, DNA damage and the Y chromosome. *Reproduction,* v.122, p.497-506, 2001.

Avenado C, Oehninger S. DNA fragmentation in morphologically normal spermatozoa; how much should we be concerned in the ICSI era? *J Androl*, 2010. Publ ahead of print Nov 18, 2010.

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*, 9:109-115, 1988.

Bennetts LE, Aitken RJ. A comparative study of oxidative DNA damage in mammalian spermatozoa. *Mol Reprod Dev*, v.71, p.77-87, 2005.

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*, v21, p.1787-1790, 2006.

Bernadini L, Borini A, Preti S, Conte N, Flamigni C, Capitanio GL, Venturini PL. Study of aneuploidy in normal and abnormal germ cells of fertile and infertile men. *Hum Reprod*, v.13, p.3406-3413, 1998.

Betts DH, King WA. Genetic regulation of embryo death and senescence. Theriogenology 2001;51:171-191,

Chan PJ, Seraj IM, Kalugdan TH, King A. Evidence for ease of tranmission of human papillomavirus DNA from sperm to cells of the uterus and embryo. *J Appl Reprod Genet*, v.13, p.516-519, 1996.

Chenoweth PJ. Genetic sperm defects. *Theriogenology*, v. 64, p.457-468, 2005.

Christopher-Hennings J, Nelson EA, Nelson JK, Rossow KD, Shivers JL, Yaeger MJ, Chase CCL, Gardano RA, Collins JE, Benfield DA. Identification of porcine reproductive and respiratory virus in semen

and tissues from vasectomised and non-vasectomised boars. *Vet Pathol*, v.23, p.260-267, 1998.
Colas G. Factors affecting the quality of ram semen. In: Haresign W (Ed.). *Sheep production*. London: Butterworths, 1983. p.453-465.
Courot M, Colas G. The role of the male in embryonic mortality. In: Sreenan JM, Diskin MG (Ed.). *Embryonic mortality in farm anmals*. Boston: Martinus Nijhoff, 1986. p.195-206.
Cottral GE, Gailiunas P, Cox BP. Foot-and-mouth disease virus in semen of uls and its transmission by artificial insemination. *Arch Virusforsch*, v.23, p.362-377, 1968.
Diaz-Garcia FJ, Herrara-Mendoza AP, Giono-Cerezo S, Guerra-Infante FM. Mycoplasma hominis attaches to and locates intracellularly in human spermatozoa. *Hum Reprod*, v.21, p.1591-1598, 2006.
Duranthon V, Renard JP. The developmental competence of mammalian oocytes: A convenient but biologically fuzzy concept. *Theriogenology*, v.55, p.1277-1289, 2001.
Erenpreiss J, Spano M, Erenpreisa J, Bungum M, Giwercman A. Sperm chromatin structure and male fertility: biological and clinical aspects. *Asian J Androl*, v.8, p.11-29, 2006.
Evenson DP, Darzynkiewicz Z, Melamed MR. Relation of mammalian sperm chromatin heterogeneity to fertility. *Science*, v.210, p.1131-1133, 1980.
Flowers WL. Increasing fertilization rate of boars: Influence of number and quality of spermatozoa inseminated. *J Anim Sci*, v.80, p.E47-E53, 2002.
Foster NM, Alders MA, Luedke AJ, Walton TE. Abnormal virus-like particles in spermatozoa from bulls latently infected with bluetongue virus. *Am J Vet Res*, v.4, p.1045-1048, 1980.
Gadea J, Matas C. Sperm factors related to in vitro penetration of porcine oocytes. *Theriogenology*, v.54, p.1343-1357, 2000.
Gledhill BL. Studies on the DNA content, dry mass and optical area of bull spermatozoa heads during epididymal maturation. *Acta Vet Scand*, v.7, p.131-142, 1966.
Guerin B, Pozzi N. Viruses in boar semen: detection and clinical as well as epidemiological consequences regarding disease transmission by artificial insemination. *Theriogenology*, v.63, p.556-572, 2005.
Hammitt DG, Aschenbrenner DW, Williamson RA. Culture of cytomegalovirus from frozen-thawed semen. *Fertil Steril*, v.49, p.554-557, 1988.
Hillery FL, Parrish JJ, First NL. Bull specific effect on fertilization and embryo development in-vitro. *Theriogenology*, v.33, p.249, 1990. Abstract.
Howard TH, Bowen RA, Pickett BW. Isolation of bluetongue virus from bull semen. In: Barber TL, Jochim M (Ed.). *Bluetongue and related orbiviruses*. New York: Alan R. Liss, 1985. p.127-134.
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*, v.13, p.372-375, 1998.
Karabinus D, Vogler CJ, Saacke RG, Evenson DP. Chromatin structural changes in bovine sperm after scrotal insulation of Holstein bulls. *J Androl*, v.18, p.549-555, 1997.
Krausz C, Quintana-Murci L, Barbaux S, Siffroi J-P, Rouba H, Delafontaine D, Souleyreau-Therville N, Arvis G, Antoine JM, Erdei E, Taar JP, Tar A, Jeandidier E, Plessis G, Bougeron T, Dadoune J-P, Fellous M, McElreavy K. A high frequency of Y chromosome deletions in males with nonidiopathic infertility. *J Clin Endocr Metab*, v.84, p.3606-3612, 1999.
Lai YM, Lee JF, Huang HY, Soong YK, Yang FP, Pao, CC. The effect of human papillovirus infection on sperm cell motility. *Fert Steril*, v.67 p.1152-1155, 1997.
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*, v.11, p.1942-1946, 1996.
Lewis SEM, Aitken RJ. DNA damage to spermatozoa has impacts on fertilization and pregnancy. *Cell Tiss Res*1996., v.322, p.33-41, 2005.
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*, v.19, p.192-194, 1992.
Miller D, Hrudka M, Cates WF, Mapletoft R. Infertility in bull with a nuclear sperm defect. *Theriogenology*, v.17, p.611-621, 1982.
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*, v.71, p.199-204, 1993.
Osburn B. The impact of bluetongue virus on reproduction. *Comp Immun Microbil Infect Dis*, v.17, p.189-196, 1994.
Piomboni P, Baccetti B. Spermatozoon as a vehicle for HIV-1 and other viruses: a review. *Mol Reprod D*ev, v.56, p.238-242, 2000.
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*, v.40, p.497-508, 1993.

Ramirez-Mandoza 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*, v.117, p.237-252, 1997.
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*, v.63, p.1041-1048, 2000.
Rosenbusch B, Strehler E, Sterzik K. Cytogenetics of human spermatozoa: Correlations with sperm morphology and age of fertile men. *Fertil Steril*, v.58, p.1071-1073, 1992.
Saacke RG, Bame J, Vogler CJ, Nadir S, Mullins J. Association of sperm nuclear vacuoles with failure of sperm to sustain embryonic development. *J Anim Sci*, v.70, suppl. 1, p.256, 1992.
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*, v.60-61, p.663-677, 2000.
Salamon S, Maxwell WMC. Storage of ram semen. *Anim Reprod Sci*, v.2, p.77-111, 2006.
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*, v.35, p.256-260, 1952.
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*, v.6, p.182-194, 2001.
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 Fertil*, v.82, p.567-574, 1988.
Schneider CS, Ellington JE, Wright RW Jr. Effects of bulls with different field fertility on in-vitro embryo cleavage and development using sperm co-culture systems. *Proc Soc Theriogenol*, p.262, 1999.
Smorag Z, Bocheneck M, Wojdan Z, Sloniewski K and Reklewski Z. The effect of sperm chromatin stucture on quality of embryos derived from superovulated heifers. *Theriogenology*, v.53, p.201, 2000. Abstract.
Stroble KA, Stewart TS, Krisher RL. Duration of crypreservation has no effect on fertilizing ability of boar spermatozoa. *Theriogenology*, v.59, p.212, 2003. Abstract.
Sur J-H, Doster AR, Christian JS, Galeota JA, Wills RW, Zimmerman JF, Osorio FA. Porcine reproductive and respiratory syndrome virus replicates in testicular germ cells, alters spematogenesis and induces germ cell death by apoptosis. *J Virol*, v.71, p.9170-9179, 1997.
Thundathil J, Meyer R, Palasz AT, Barth AD, Mapletoft RJ. Effect of the knobbed acrosome defect in bovine sperm on IVF and embryo production. *Theriogenology*, v.54, p.921-934, 2000.
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*, v.40, p.207-1219, 1993.
Walters AH, Eyestone WE, Saacke RG, Pearson RE, Gwazdauskas FC. Bovine embryo development after IVF with spermatozoa having abnormal morphology. *Theriogenology*, v.63, p.1925-1937, 2005.