Reviews of Reproduction (2000) **5**, 46–52

Cryopreservation of semen from domestic livestock

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Fifty years after the first successful cryopreservation of spermatozoa, the technique is an integral part of the cattle breeding industry but has failed to establish itself commercially in the production of other breeds of domestic livestock. New assessment techniques have shown that the ejaculate consists of a heterogeneous population of cells, which achieve their full fertility potential at different rates within the female tract and thus maximize the chances of a fertile spermatozoon successfully combining with an egg. It is becoming apparent that the freeze–thaw process results in a more homogeneous cell population, which may be functionally compromised. One aspect of sperm function that has been demonstrated to be affected by cryopreservation is the process of capacitation. Chlortetracycline staining has shown that frozen–thawed spermatozoa undergo an accelerated 'capacitation-like' process which has implications for their interaction with the female tract, ability to establish sperm reservoirs *in vivo* and hence for their life expectancy after insemination. In addition to heterogeneity within the ejaculate, there is increasing evidence for variation between individuals in the success of sperm freezing. Post-thaw sperm survival may be consistently poor for certain individual animals even though pre-freeze parameters appear normal. The mechanisms that may underlie such differences in cryosensitivity remain unclear. A greater role for the use of frozen semen in livestock production can come only from an improvement in the preservation of the functional competence of the cryopreserved spermatozoon after insemination into the female tract.

It is just over 50 years since Polge, Smith and Parkes described the use of glycerol to freeze spermatozoa (Polge *et al*., 1949), and is perhaps a good time at which to consider the progress made in semen cryopreservation over the last half century. In 1970, in a paper for the *Veterinary Record* entitled 'Fertilizing capacity of frozen boar semen following surgical insemination' (Polge *et al*., 1970), the authors stated:

> Over 20 years have now passed since the first successful experiments on freezing semen in the presence of glycerol (Polge *et al*., 1949) but so far it is only in the artificial insemination of cattle that it has been possible to exploit this technique and to apply it on a large scale.

At the time of writing, it could hardly have been foreseen that at the end of the 1990s that the situation would be virtually unchanged.

The first calf produced from cryopreserved spermatozoa was born in 1951 (Stewart, 1951) and the technique very rapidly established itself thereafter. Today the modern cattle industry worldwide is based on the use of artificial insemination and frozen semen. Top sires can produce up to 60 000 doses of semen per year, and thus cryopreservation has allowed exploitation of superior sires and achieved rapid, large-scale genetic improvement in cattle stocks coupled with a reduction in disease transmission.

Frozen semen should have had the same potential for other livestock species and early successes (Table 1) initially held the promise of an equally important role for freezing in other species, for example in commercial pig and sheep production. However, it rapidly became apparent that many of these initially positive results were not readily reproducible and that the advances of the cattle industry would prove harder to repeat elsewhere.

The use of frozen semen for artificial insemination has some obvious advantages, but if it is to be of commercial value, procedures must be straightforward and results must be at least comparable to those achieved by natural mating. For cattle these conditions have been largely met and success rates with frozen semen have matched rates from insemination with fresh semen and from natural mating, but for sheep, pigs and horses the situation is very different.

Artificial insemination of pigs has been and remains a growth area; in 1998–1999 approximately 40% of pig production in the UK was by artificial insemination, compared with about 11% in 1990. However, although frozen semen is used to a very limited extent for genetic improvement, for conservation of rare or valuable genetic material and for overseas import–export, day-to-day commercial use is effectively non-existent. Low farrowing rates and small litter sizes have discouraged the use of frozen semen, while the relatively long shelf-life of extended boar semen (approximately 7 days) at ambient temperatures has allowed many of the logistical problems involved in collecting and distributing semen for insemination to be solved without the need for cryopreservation.

The situation in the sheep industry differs in that it is the technique of artificial insemination that is restrictive with very low success rates for trans-cervical insemination. When artificial insemination is used for sheep, it is generally laparoscopic and frequently utilizes frozen semen and good success rates are obtained. However, surgical insemination is obviously a veterinary procedure, is expensive and has been criticised on

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Table 1. First recorded offspring produced by artificial insemination using frozen–thawed semen in different species

Species	Date	Reference
Cows	1951	Stewart
Sheep	1967	Salmon and Lightfoot
Pigs	1957	Hess *et al.*
Horses	1957	Barker and Gandier
Humans	1953	Bunge and Sherman

Table 2. Membrane permeability parameters for spermatozoa from different species

Species	Lp[a] (μm min^{-1} atm^{-1})	Ea[b] (kcal mol^{-1})	Reference
Boars	1.0	–	(Gilmore *et al.*, 1996)
Bulls	10.8	3.0	(Watson *et al.*, 1992)
Fowl	2.1	4.4	(Watson *et al.*, 1992)
Humans	2.9	1.9	(Curry *et al.*, 1994)
Rabbits	0.6	17.8	(Curry *et al.*, 1995)
Rams	8.5	1.1	(Curry *et al.*, 1994)

[a]Hydraulic conductivity; [b]activation energy.

welfare grounds. Taken together, these factors have severely restricted its use.

Artificial insemination of horses has also been limited particularly in the UK where thoroughbred breeding, which constitutes a major part of the British equine industry, does not permit its use. It is also probably true to say that there is a general prejudice that is still to be overcome particularly against the use of frozen semen. There has been much more extensive use in parts of Europe, for example the Netherlands and Germany, where there is a greater interest in breeding sport horse types. Success rates with frozen semen have been good in the hands of experienced operators, although generally below those of natural coverings. Breeding of horses fundamentally differs from that of pigs, sheep or cattle which are managed on a herd basis and artificial insemination and frozen semen potentially allows the use of top grade stallions to mare owners; however, use has been limited by the higher veterinary costs incurred. The widespread availability of chilled semen, which remains viable for up to 72 h, has, as with the boar, alleviated pressure to improve success rates with cryopreserved semen.

It is not the aim of this review to consider in detail the differences in sperm sensitivity to freezing damage between species or to describe different freezing protocols. A large body of empirical work has resulted in optimization of dilution and freeze–thawing methods for different species and this has been comprehensively covered by a number of recent reviews (Watson, 1990; Bwanga, 1991; Pickett and Amann, 1993; Salamon and Maxwell, 1995). Rather it is the intention here to consider why the use of frozen bull spermatozoa has been so much more successful than that of other species. Is there an intrinsic difference in bull spermatozoa compared with those of the other domestic livestock species or can the success achieved in cattle be accounted for by general differences in reproductive physiology and breeding management systems?

Evidence over the last fifty years has suggested that in all species so far investigated approximately 50% of cells survive the freezing process, as measured by indicators such as membrane integrity or motility. There are significant differences between species in sperm biophysical parameters, such as cell surface area, cell volume, water volume and membrane permeability to water (Table 2; Curry *et al.*, 1996). These biophysical parameters have been used to produce a theoretical model for optimal freezing regimens in an attempt to move on from the empirical approach that has dominated the development of sperm cryopreservation over the last 40 years. However, to date, established models that have proved valuable for a range of other cell types have been found to be less appropriate for spermatozoa (Curry *et al.*, 1994). Despite these observed differences, it remains the case that under optimized conditions there is little apparent overall difference in the cryosurvival rates obtainable for spermatozoa from the different livestock species.

Cryoprotectants

The discovery of the cryoprotective actions of glycerol allowed freezing of spermatozoa and although hundreds of potential cryoprotective agents (CPAs) have subsequently been examined, glycerol has remained, almost without exception, the cryoprotectant of choice for spermatozoa from all species. However, despite extensive experimentation to optimize its use, the basis of the cryoprotective properties of glycerol and precisely why it should be more effective than other CPAs remains unclear. Glycerol is a permeating cryoprotectant able to cross the cell membrane and the recent identification of the water channel protein Aquaporin 7 (AQP7) which also facilitates glycerol transport, at the late spermatid stage in the rat testis (Ishibashi *et al.*, 1997) may provide a specific route for glycerol entry into spermatozoa. Previous studies have shown that spermatozoa from many of the domestic species have a high permeability to water and a low activation energy (Table 2) and an insensitivity to inhibition by mercuric chloride (Watson *et al.*, 1992; Curry *et al.*, 1994; Gilmore *et al.*, 1996), membrane characteristics consistent with the presence of the AQP7 channel. It has been difficult to ascribe a physiological role to the high water permeability of the sperm membrane and if AQP7 is found to be widely expressed amongst the domestic species it may be that the transport of glycerol and not of water is its primary function. Unlike the other species examined, rabbit spermatozoa have a relatively low water permeability and a high activation energy, parameters associated with lipid phase rather than channel-mediated water transport (Curry *et al.*, 1995). The rabbit is also one of the few exceptions in which dimethyl sulfoxide (DMSO) and not glycerol is the preferred cryoprotectant and it is possible that this might be due to a lack of the AQP7 glycerol transporter, although currently there is no direct evidence to support this. The cryoprotective effects of glycerol are most evident at higher concentrations but have to be

balanced against glycerol toxicity, which is detrimental at concentrations much higher than 0.5 mol l^{-1}. The sensitivity of spermatozoa to these toxic effects varies with species. Susceptibility of boar spermatozoa to acrosomal damage at relatively low glycerol concentrations, for example, may explain the poor fertility of frozen–thawed boar spermatozoa compared with more resistant bull spermatozoa. Adverse effects need not necessarily be immediately apparent; glycerol has a marked antifertility effect on fowl spermatozoa (Lake *et al*., 1980) without causing obvious morphological damage or impairing motility. Although mammalian spermatozoa do not appear to exhibit this effect to the same extent, without a clear understanding of the mechanisms involved, functional damage of this type cannot be completely discounted.

Heterogeneity of spermatozoa

Fertilization requires the spermatozoa and egg to meet in the ampulla of the oviduct, both having completed crucial maturation processes. For induced ovulators such as rabbits or cats, ovulation is triggered by mating and a fixed interval between deposition of spermatozoa in the female tract and release of an egg allows maturation of male and female gametes to be synchronized. However, for many species the situation is more complex. The female may be receptive to mating during a prolonged oestrous period and ovulation may occur at any time within this period, either before or after mating. With variable insemination and ovulation times, spermatozoa must be able to survive within the female tract for periods up to several days and fully functionally spermatozoa competent to fertilize an egg must be available within the oviduct when ovulation occurs. One way in which this could be achieved is by spreading the individual acquisition of fertilizing competence over a period of time by having a heterogeneous population of spermatozoa. Heterogeneity of spermatozoa would ensure a greater potential for fertilizing an oocyte at some unpredictable interval after ejaculation. A heterogeneous sperm population within the female tract implies a heterogeneous ejaculate, and there is evidence that this is the case. Spermatozoa acquire fertilizing competence in stages during passage through the epididymis and subsequently through the female tract. Epididymal transport occurs over several days by peristaltic-like contractions; however, this transport need not be strictly linear. Intermixing would generate subpopulations of spermatozoa differing in age after spermiation and probably in degree of maturation.

Heterogeneity within the ejaculate is clearly important for successful fertilization after natural mating, but could also be a significant factor in the use of frozen–thawed spermatozoa for artificial insemination. Amann *et al*. (1993) described epididymal maturation in terms of the spermatozoa acquiring a 'combined effective amount' of different individual attributes which taken together render the spermatozoa fertile. This concept of 'combined effective amount' is used by Amann *et al*. (1993) to emphasize that acquisition of fertilizing ability does not result from a single maturational event but is a consequence of interactions between a sequence of multiple steps and that the relative importance of any individual step could change under different conditions. Treatments imposed on spermatozoa can alter their 'combined effective amount' of attributes and it is possible that a specific attribute is limiting at one stage during the life of the spermatozoon or at a particular site, but will be in excess at another. Attributes necessary for success will be different for *in vitro* fertilization, intrauterine artificial insemination, intra-cervical insemination or natural mating. Use of cryopreserved rather than fresh spermatozoa can also affect the blend of attributes required in the 'combined effective amount'. Although some of the necessary attributes for fertile spermatozoa have been identified, others remain unknown, as does the effective combination required in these different circumstances. This level of complexity and the number of unknown factors make it extremely difficult to assess the potential fertility of spermatozoa effectively both within the ejaculate and after freezing and thawing.

Even if the nature and importance of sperm subpopulations are still unclear, it is apparent that there are changes in these subpopulations following the freeze–thaw process. Examination of post-thaw spermatozoa with techniques such as partitioning in aqueous two-phase systems to detect subtle differences in surface properties (Harrison *et al*., 1992) has demonstrated that heterogeneity is severely diminished after the freeze–thaw process (Ollero *et al*., 1998). Loss of heterogeneity could be accounted for in one of two ways; the freezing process might differentially affect a particular population of cryo-sensitive cells, thereby effectively selecting for a cryo-resistant, if not necessarily optimally fertile, population. Alternatively the freeze–thaw process might affect all cells to such an extent that pre-existing more subtle differences are masked giving the appearance of a more homogeneous population. With only a limited understanding of the nature and extent of the cryo-injury suffered by individual cells, it is difficult to decide between these hypotheses and the true situation may lie between the two, with a sensitive population of cells at the severe end of a spectrum of damage effecting all cells within the ejaculate. Future studies might usefully involve evaluation of the heterogeneity of spermatozoa in an ejaculate with multivariate techniques, an approach that has been used successfully for looking a different patterns of sperm motility following computer-assisted motility analysis (Davis and Siemers, 1995; Abaigar *et al*., 1999).

Capacitation and cryopreservation

The early successes with freezing spermatozoa were almost contemporaneous with another key discovery in reproductive biology. Austin (1951) and Chang (1951) independently identified the need for ejaculated spermatozoa to undergo a process of capacitation before they were competent to fertilize an egg. However, it is only in the last few years that the relevance of capacitation to successful sperm cryopreservation has started to be fully appreciated. Capacitation has been described in numerous studies (Cohen-Dayag and Eisenbach, 1994; Harrison, 1996) and it is known that spermatozoa must have completed the process in order to undergo an acrosome reaction. Therefore, the acrosome reaction has been a useful endpoint for determining whether cells are fully capacitated. The rate and extent of the changes that precede the acrosome reaction have been more difficult to investigate because there are no observable changes in morphology and because spermatozoa within an ejaculate do not capacitate synchronously. More

recently the fluorescent antibiotic chlortetracycline (CTC) has been used to study capacitation in a number of species. Three different CTC staining patterns have been characterized (Fig. 1), an F pattern associated with uncapacitated cells, a B pattern for cells that have at least partially completed capacitation but have not acrosome reacted and the AR pattern characteristic of acrosome-reacted cells. The F, B and AR patterns were originally described for mouse spermatozoa (Ward and Storey, 1984) and have since been identified in a number of species including bulls (Fraser *et al*., 1995; Cormier *et al*., 1997), boars (Wang *et al*., 1995; Mattioli *et al*., 1996; Maxwell and Johnson, 1997), rams (Perez *et al*.,1996; Gillan *et al*., 1997) and stallions (Varner *et al*., 1987). Perez *et al.* (1996) used CTC staining to demonstrate an accelerated rate of capacitation *in vitro* for frozen–thawed ram spermatozoa. After incubation for 2 h after thawing, 39% of cells showed the B pattern compared with only 16% of fresh spermatozoa incubated for the same period. Gillan *et al*. (1997) reported similar findings, with 61% (F), 18% (B) and 21% (AR) for fresh ram spermatozoa compared with 7.2% (F), 66% (B) and 26% (AR) for frozen–thawed semen. After incubation for 6 h at 37°C, the fresh spermatozoa showed a large increase in the B pattern from 18 to 54% and a smaller increase in the AR pattern from 21 to 41%. The frozen–thawed spermatozoa in contrast showed a fall in the B pattern from 66 to 35%, with a concomitant increase in the AR pattern from 21 to 64%. In both studies the process of freezing and thawing increased the number of spermatozoa showing the capacitated B pattern but had little effect on the numbers of acrosome-reacted spermatozoa.

Fuller and Whittingham (1997) described a similar effect in mice, not with frozen–thawed spermatozoa but with cells slowly cooled to 4°C and then rewarmed. The cooled cells had more than 80% B pattern compared with only 20% in the control group. The effect of slow cooling to above zero temperatures has also been observed with boar spermatozoa (Maxwell and Johnson, 1997). Freeze–thawing led to a decrease in the F staining pattern and an increase in the B staining pattern and cooling to 5°C had a greater effect than cooling to 15°C with 30.4% (F), 48.5% (B), 21.1% (AR) at 5°C compared with 56.1% (F), 32.6% (B), 11.5% (AR) at 15°C. This relationship between the number of B pattern cells and the extent of cooling has more recently been confirmed by Green and Watson (1999). Changes in CTC staining patterns have also been reported for bull spermatozoa (Cormier *et al*., 1997): after either cryopreservation or cooling to 4°C there is a similar increase in the capacitated B staining pattern.

Taken together these results indicate that in the domestic species examined to date, cryopreservation, or even simple cooling, induces a change in the spermatozoa such that their CTC staining properties alter from those characteristic of uncapacitated spermatozoa to those of capacitated but acrosome-intact cells. This is a somewhat carefully worded conclusion as the precise basis of the changes in CTC staining patterns during capacitation remains unclear. Without a better understanding of the processes it is not possible to state the extent to which the changes observed after cooling and cryopreservation are the same as those seen with *in vivo* or even *in vitro* capacitation. There is some preliminary evidence from studies with boar spermatozoa of differences between the phosphorylated intermediates that can be detected after *in vitro* capacitation and those detectable after slow cooling (C. Green, personal communication).

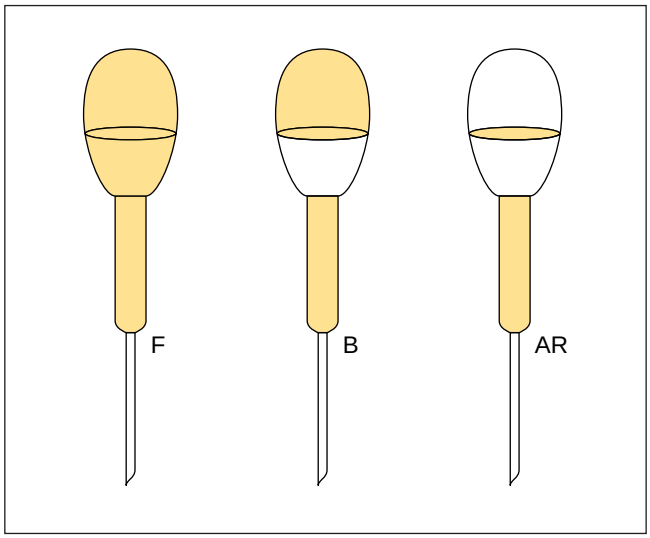

Fig. 1. Chlortetracycline fluorescent staining patterns for uncapacitated (F), capacitated (B) and acrosome-reacted (AR) spermatozoa.

Evidence that frozen–thawed spermatozoa are capacitated comes from functional assays such as the hamster egg penetration test and *in vitro* fertilization studies. Unlike fresh spermatozoa which require a period of preincubation and only show their maximal penetration rate after 24 h, cryopreserved human spermatozoa show their highest penetration of zona-free hamster eggs immediately after thawing and the penetration rate declines over 48 h thereafter (Critser *et al*., 1987). Other studies have shown that frozen–thawed bull (Wheeler and Seidel, 1986) and ram (Garde *et al*., 1993) spermatozoa can also penetrate eggs more rapidly than unfrozen spermatozoa. Studies in Maxwell's laboratory, although finding no difference in fertilization rates, reported that sheep oocytes fertilized with frozen–thawed spermatozoa *in vitro* had reached a more advanced stage of development after co-incubation for 20–22 h than had comparable oocytes fertilized with fresh spermatozoa (Gillan *et al*., 1997). These studies all appear to indicate that the changes in CTC staining pattern are accompanied by the achievement of some functional fertilizing capacity in frozen–thawed spermatozoa. This functional change needs to be recognized and taken into account when performing artificial insemination with frozen–thawed spermatozoa.

Lifespan of spermatozoa

It was recognized at an early stage in the history of semen preservation that cryopreserved spermatozoa had a much shorter lifespan within the female reproductive tract compared with fresh semen. This finding has subsequently been confirmed in a number of studies and for a range of species (Mattner *et al*., 1969; Hawk 1983). Capacitation does not produce a population of cells in a defined and stable 'activated' state but is a continuing process of positive destabilization and as such will eventually lead to cell death (Harrison, 1996). On reaching a threshold level of destabilization, the spermatozoa exhibit hyperactivated

motility and are capable of undergoing acrosome reaction in response to an appropriate signal and at this point can be classed as capacitated. However, once it begins the destabilization process will continue beyond the threshold level with progressive degeneration of membrane function until the spermatozoa is unable to maintain cell integrity. Thus capacitation leads inexorably to cell death for the non-fertilizing spermatozoa. Therefore, if frozen–thawed spermatozoa exhibit an accelerated rate of capacitation, this might account for a shortened lifespan for these cells. In many mammalian species, including cows (Hunter *et al*., 1991), pigs (Hunter, 1983) and sheep (Hunter, 1983), spermatozoa introduced into the female tract accumulate in a sperm reservoir in the caudal isthmus of the oviduct. Spermatozoa are maintained in the distal oviduct until about the time of ovulation when, in response to some, as yet unknown, signal they are progressively released and move up to meet the egg in the ampulla. Studies *in vitro* have demonstrated that attachment to oviductal epithelial cells prolongs the lifespan of spermatozoa (Suarez *et al*., 1991; Pollard *et al*., 1991). Furthermore, studies using pig oviductal epithelial cells in culture (Fazeli *et al*., 1999) have shown that the oviductal epithelium preferentially binds uncapacitated spermatozoa; similar findings have been reported for bovine (Lefebvre and Suarez, 1996) and equine (Thomas *et al*., 1995) spermatozoa. If capacitation diminishes the ability of spermatozoa to bind to the oviductal epithelium, the change in status described for frozen–thawed spermatozoa may interfere with the establishment of the isthmic sperm reservoir. Failure to establish the normal sperm–epithelial interactions may contribute to the reduced fertility and shorter lifespan of the frozen–thawed spermatozoa. Poor fertility associated with a compromised ability to survive within the female tract and to establish sperm reservoirs can be overcome by carefully timing insemination with frozen–thawed semen to coincide closely with ovulation. An increased need for precise timing is, to a greater or lesser extent, part of all insemination procedures but is of particular importance in, for example, the mare in which successful insemination with frozen–thawed semen is dependent upon follicular monitoring so that insemination can occur within 6 h of ovulation. Insemination can also be used to overcome the natural barriers to sperm transport *in vivo*. Semen deposition after mating, depending on species, can either be intra-vaginal (for example cow) or intra-uterine (for example sow). The cervix, between the vagina and the uterus and the utero–tubal junction, between the uterus and the oviduct, act not only as passive barriers that the spermatozoa must cross but also as filters selecting which spermatozoa will proceed to the next section of the tract. Intra-uterine insemination bypasses the cervical barrier and intra-tubal insemination bypasses both the cervix and the utero–tubal junction but in doing so may allow passage to spermatozoa that would otherwise have been blocked.

If, under conditions *in vivo*, capacitated spermatozoa are present only within the oviduct and if the freeze–thawing results in a capacitated sperm population, it might be predicted that the oviduct would be the most effective site to place frozen–thawed spermatozoa (Fig. 2). This supposition is borne out by evidence in sheep and pigs in which surgical intra-tubal insemination has yielded good results in marked contrast to trans-cervical insemination with frozen–thawed spermatozoa.

Selection for 'good freezers'

Variation between individuals in the extent to which their spermatozoa are damaged by freeze–thawing has been widely reported. Human semen donors have, for example, routinely been categorized as 'good' or 'bad freezers', although the evidence for such differences is often somewhat anecdotal. The concept of poor freezers suggests that cryosurvival is not necessarily related to the observed quality of the semen sample such that, for certain individuals with apparently good pre-freeze sperm parameters post-thaw survival is consistently poor.

Stallions have been particularly noted for a high degree of individual variation in sperm cryosurvival. It has been estimated that 25% of stallions produce semen that freezes well, 50% freeze acceptably and 25% freeze poorly (Pickett and Amann, 1993). Similarly, large individual differences have been described between boars. For example, in a recent study it has been possible to assign individual boars into good, average and poor freezer groups on the basis of their post-thaw sperm viability using a system of multivariate pattern analysis (Thurston *et al*., 1999).

The mechanisms underlying differences in cryosensitivity between different individuals have yet to be elucidated, but there is some evidence for physiological differences between spermatozoa from individuals of the same species. Harrison *et al.* (1996) reported that the stimulatory effects of bicarbonate on the process of capacitation differ among individual boars. However, such differences could be genetic or non-genetic in origin. A role for a genetic component in the overall effect is suggested by the strain differences in cryosensitivity recorded for mice (Nakagata and Takeshima, 1993). The positive effects of selection would also seem to point to a genetic component for 'good freezing' as a characteristic.

Individual differences have been recorded for bull semen and allowances have been made for so-called poor freezers by packaging straws with more spermatozoa or by adjusting freezing protocols for individual bulls (Parkinson and Whitfield, 1987). If there has been a significant problem with variation between individual bulls, it has been largely overcome by an ongoing selection process whereby, even though bulls have not been selected primarily on the basis of their fertility, those whose semen has proved to be subfertile have been removed from freezing programmes. The large numbers of inseminations from a single bull allow the calculation of an estimated relative conception rate (ERCR) for individual sires. ERCR is a measure of the conception rate of a service sire relative to service sires of herd-mates and is expressed as the percentage difference in conception rate from the average AI service sire. Results from the United States for Holstein and Jersey bulls over the period 1995–1997 ranged from –19% to +11% (data from Dairy Records Management Systems, USA). Thus service to the most fertile bull resulted in a calf 11% more often than did service to an average bull, and conversely the least fertile bull produced a calf 19% less often. Acceptable fertility was judged to be within the range ± 3%, and 65% of bulls fell within this category. Availability of statistics such as ERCR makes it possible to calculate an additional monetary value for semen from a sire with a high ERCR score (Pecsok *et al*., 1994), with in practice estimated increases in value per

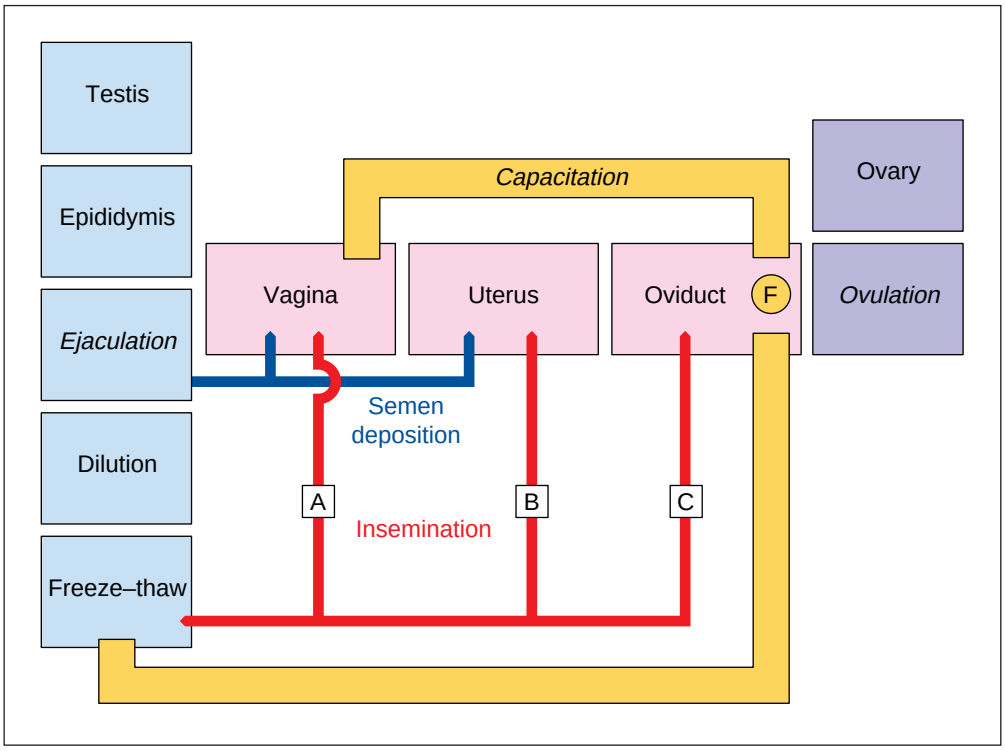

Fig. 2. Schematic diagram of the stages in gamete production, transport and fertilization. Semen deposition in the course of mating may be intra-vaginal or intrauterine. Frozen–thawed spermatozoa may be inseminated into the vagina (A) into the uterus trans-cervically (B) or laparoscopically into the oviduct (C).

unit semen of between $0.6 and $5.01. The recorded effect of service sire on conception rate is small but when other traits are equal, fertility may be an important secondary trait. In this way there is an increased incentive to use bulls of high fertility with frozen semen, that is, bulls with good functional cryosurvival. For other domestic species, the numbers of inseminations from an individual sire are generally too low to provide reliable information for selection on fertility. Sire selection is predicated from other attributes, such as meat or wool production in pigs and sheep or speed in horses. Fertility and particularly fertility of frozen semen is not currently a primary trait to be taken into account with these species when choosing which males to use for breeding.

Conclusion

The story of semen cryopreservation has been one of apparent early success followed by decades of marginal improvement. However, it is becoming increasing clear that even when membrane-intact, motile spermatozoa are recovered after thawing, these cells are functionally very different from fresh spermatozoa. If frozen–thawed semen is to be used in the same way as fresh semen, and be able to achieve high fertility rates from intrauterine or even intra-vaginal insemination, without very careful monitoring of the female cycle, it will be necessary to substantially improve techniques for freezing–thawing. A greater understanding of the causes of cryo-injury and the effects of temperature, osmotic and oxidative stresses on sperm membranes are required in order to preserve the natural heterogeneity of a semen sample and to limit pseudo-maturational changes in the cells. There is little evidence to indicate that bull spermatozoa are unique and good reason to hope that it will not take another 50 years to fulfil the potential of frozen semen in a wide variety of species.

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