Can we use in vitro fertilization tests to predict semen fertility?

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Abstract

This presentation deals with assays based on in vitro fertilization (IVF) and related techniques such as zona pellucida (ZP) binding assays and oocyte penetration tests. These types of assays have been developed for several species of domestic animals. A description of the assays and how they have been performed in domestic animals, as well as data on the correlation between the results of assays and actual in vivo fertility are presented. Used either as single tests or in combination with other tests, this type of assay can provide valuable information about a semen donor, an insemination dose or a method of semen preservation. © 2000 Elsevier Science B.V. All rights reserved.

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1. Introduction

Before using a male for breeding purposes, it is important to know that he is fertile. In some cases, it is enough to know that he will be able to produce offspring, while in others, knowledge of a more accurate level of his fertility is desired. The latter is the case when a male is going to be used for artificial insemination (AI), serving large numbers of females. Therefore, over the years, several methods have been developed that aim at predicting male fertility. To test male fertility by mating or AI is expensive and time-consuming, and only allows a limited number of males to be tested. Laboratory methods do not suffer from these drawbacks, and to test different aspects of semen quality, several methods have been designed, some of which have been correlated to
field fertility. Among the aspects of semen quality analysed is sperm morphology (Barth, 1992), sperm motility (Kjaestad et al., 1993; Holt et al., 1997), the biochemical compounds in the semen (Hirao, 1975), presence of intact acrosomes (Correa et al., 1997), membrane integrity (Pérez et al., 1997), concentration of swim-up separated spermatozoa (Zhang et al., 1998) and the ability to spontaneously undergo capacitation (Januskauskas et al., 2000; Thundathil et al., 1999) and to undergo acrosome reaction (Whitfield and Parkinson, 1995; Januskauskas et al., 1999). However, few single sperm parameters show a significant correlation with in vivo fertility for semen samples within acceptable ranges of normality. The more sperm parameters that can be tested, the more accurate the test will be (Amman and Hammersedt, 1993). In this respect, interest has lately been focused on test methods related to the in vitro fertilization (IVF) technique.

2. IVF and related techniques

IVF has been developed and used for producing offspring in many domestic animal species (Brackett et al., 1982a; Cheng et al., 1986; Palmer et al., 1991). The in vitro technique implies in vitro maturation of oocytes (IVM), IVF of oocytes and in vitro culture (IVC) of fertilised oocytes up to the blastocyst stage. Over the years, many studies, performed mainly in cattle, have shown that the donor of the semen largely influences the outcome of both IVF and IVC (Eyestone and First, 1989; Shi et al., 1990; Shamsuddin and Larsson, 1993). This difference in in vitro fertility between semen donors led to studies designed to investigate whether there was a correlation between in vitro and in vivo fertility (i.e. Marquant-Le Guienne et al., 1990; Zhang et al., 1997). The endpoints of these studies have been cleaved oocytes and/or their development to morulae or blastocysts. In vitro fertility can also be assessed by the so-called penetration tests (Wheeler and Seidel, 1987; Tatemoto et al., 1994; Matás et al., 1996; Hay et al., 1997a; Gadea et al., 1998). In these tests, oocytes penetrated by spermatozoa and/or having visible pronuclei inside the ooplasma are the endpoints.

Assays based on the ability of spermatozoa to bind to homologous or heterologous zona pellucida (ZP) have been developed as diagnostic tests in several animal species. The result of a ZP-binding assay can be presented as the total number of spermatozoa bound to the ZP (absolute ZBA; Zhang et al., 1998). An alternative is to calculate a ZP-binding index where the binding capacity of a test semen sample is compared with that of a sample from a control animal (ZBA index; Fazeli et al., 1997; Zhang et al., 1998). The variation in binding capacity between oocytes can be avoided by using a large number of oocytes per batch and several batches per test (Zhang et al., 1995) or by performing hemi-zona assays (HZA) (Franken et al., 1993; Fazeli et al., 1997). In a HZA matching, hemi-zonae are incubated with samples of test semen and control semen.

The availability of oocytes will influence the choice of assay. To conduct tests based on IVF and IVC, one must have access to oocytes that have the potential to develop after fertilization. These oocytes are most commonly obtained from oviducts (Fukui et al., 1988; Pantke et al., 1995; Zhang et al., 1997; Lynham and Harrison, 1998), but they can also be retrieved after ultrasound-guided ovum-pickup in live animals (Fazeli et
In species such as the dog, restricted numbers of oocytes can be obtained after ovariohysterectomy (Ström Holst et al., 2000a). To guarantee that numbers of oocytes are high enough to make sperm evaluation possible, oocytes might have to be stored, either in salt solutions or recovered from ovaries that have been stored frozen (Wheeler and Seidel, 1987; Chian et al., 1991; Tatemoto et al., 1994; Ström Holst et al., 2000a). Although immature or stored oocytes do not have the potential of development after fertilization, they can be used in penetration tests or in ZP-binding assays.

Assays can also be performed using heterologous oocytes in cases where the availability of homologous oocytes is restricted. Yanagimachi et al. (1976) suggested that zona-free hamster oocytes be used to evaluate the fertilizing potential of human sperm, and they have also been used for sperm assessment in domestic animals (Bousquet and Brackett, 1982; Berger, 1989). Heterologous fertilization by spermatozoa derived from species within the genus Bos has been performed with cattle oocytes (McHugh and Rutledge, 1998), and Cox et al. (1994) demonstrated that goat spermatozoa can penetrate cattle and sheep oocytes.

Since fertilization is a process that requires several sperm functions, it seems logical to combine the outcome of different semen evaluation tests in order to achieve a better correlation between test results and in vivo fertility, as suggested and extensively discussed by Amman and Hammerstedt (1993). Hirao (1975), Wood et al. (1986) and Farrell et al. (1998), investigated the correlation between both single and combined tests of sperm function and in vivo fertility, and found that certain parameters turned out to be more valuable than others in such a combination. Studies that included results of IVF/IVC and ZP-binding as well as other test parameters were performed by Zhang et al. (1998, 1999).

The results of studies on the correlation between tests and in vivo fertility will vary depending on how in vivo fertility is defined and how field fertility data are obtained and presented. In bovine studies, the results of tests obtained with an ejaculate have been compared with in vivo fertility data from the same ejaculate (Zhang et al., 1997, 1998, 1999) or to the lifetime fertility of the bull (Schneider et al., 1999). However, it is important to remember that male fertility usually changes over time; thus, the test results obtained with a given ejaculate might not be representative of lifetime fertility. A comparison between in vivo and in vitro fertility requires that data on in vivo fertility be accurate. In most species, the number of females served by a single male is too small to allow reliable data to be obtained. One exception is the bovine, where a bull’s in vivo fertility is most often expressed as non-return rates (NRRs) after AI of a large number of females. It is well known that both maternal and environmental factors influence field fertility. One way to avoid the influence of these factors is to use NRRs that have been corrected for the influences of season, area, inseminator and parity (Zhang et al., 1999). Other factors that can influence the outcome of correlation studies are the range in fertility among the males. The chance of finding a significant correlation between in vivo fertility and the outcome of an assay seems to be higher when the variation in field fertility is large (Linford et al., 1976; Zhang et al., 1999). Another factor that has to be considered in the in vitro assessment is the variation between ejaculates. There are differences in both in vivo and in vitro fertility among ejaculates collected within a
narrow time span from the same bull (Zhang et al., 1997). Therefore, a representative number of ejaculates has to be tested and the numbers tested per bull has varied between three and eight (Otoi et al., 1993; Zhang et al., 1997).

3. Results of assays based on ZBA, penetration tests and IVF/IVC in domestic animals

The importance of determining the exact level of the fertility that a male can achieve is most pronounced in the bovine owing to the fact that a single male is, via AI, used to serve many females. This can largely explain why interest in finding accurate methods for testing semen fertility has been greatest in this species. In addition, due to the high degree of refinement of bovine IVF and its frequent use, more studies have been carried out on the correlation between in vivo and in vitro fertility in this species than in other domestic animals.

3.1. Bovine

In the bovine, the ZBAs comprise absolute ZBA as well as ZBA index and hemizona assays (Fazeli et al., 1997; Zhang et al., 1998). When semen was tested using HZA, the test correlated significantly with field fertility (Fazeli et al., 1997). However, neither Fazeli et al. (1997) nor Zhang et al. (1998) found a significant correlation between ZBA index and field fertility, probably owing to the small number of oocytes per assay while testing of a large population of bulls and ejaculates in an absolute ZBA, using large numbers of oocytes per tested batch, resulted in significant correlation with NRRs (Zhang et al., 1998).

A different approach to testing bull spermatozoa in a penetration test was used by Henault and Killian (1995). Spermatozoa from bulls differing in their in vivo fertility were labelled with bull-specific fluorescent dyes, so that their relative abilities to penetrate the same oocytes could be assessed. The results showed that spermatozoa from highly fertile bulls were superior to spermatozoa from low-fertile bulls when it came to penetrating the oocyte.

Although a correlation between IVF and IVC results and in vivo fertility has not been found in all investigations (Ohgoda et al., 1988; Schneider et al., 1999), the findings of several studies support the concept of using IVF to assess the fertilizing ability of bull semen (Hillery et al., 1990; Marquant Le Guienne et al., 1990; Shamsuddin and Larsson, 1993; Zhang et al., 1997). Zhang et al. (1997) found a significant positive correlation between cleavage rates in vitro and in vivo fertility expressed as NRRs after AI. The correlation was higher between the cleavage rate in vitro and NRRs than between blastocyst rates and NRRs, most likely because blastocyst formation is much more dependent on conditions prevailing during embryo culture than cleavage is.

Several factors have been demonstrated to influence IVF results within the same bull. For example, the way in which spermatozoa are selected for IVF (Percoll or swim-up separation) is important (Parrish et al., 1995). Kroetsch and Stubbings (1992) showed that bull had no effect on IVF results when large numbers of spermatozoa were used but
had a clear effect when the sperm concentration was lowered. Further, the method used to induce capacitation in vitro can in some bulls influence the IVF outcome (Saeki et al., 1995).

Most studies done so far in which a relation between fertility after AI and in vitro assays has been found have been retrospective; i.e. they were done with semen with known and often wide spread fertility after AI (Zhang et al., 1998). In an effort to try to predict the fertility of semen from young AI bulls entering a national AI-breeding programme, a prognostic test was performed. A number of tests, including IVF and absolute ZBA, were combined to assess the fertility of the donor and to calculate expected NRRs (Zhang et al., 1999). Actual NRRs later obtained for the same bulls (i.e. field fertility) were strongly related to the calculated NRRs. These results indicate that a combination of laboratory tests can be used to determine semen quality and predict a bull’s potential fertility.

3.2. Porcine

Large numbers of oocytes can be obtained from pig ovaries collected after slaughter for assays based on IVF techniques. The oocytes have been used in penetration tests either before or after maturation (Ivanova and Mollova, 1993; Martínez et al., 1993) or used, fresh or stored, in ZP-binding assays (Fazeli et al., 1995a; Lynham and Harrison, 1998).

As is the case in bovines, the boar has been demonstrated to affect the outcome of IVF (Xu et al., 1996; Long et al., 1999). Correlations between test results and the in vivo fertility of boar semen have been measured in a number of studies. Ivanova and Mollova (1993) demonstrated that in vitro matured porcine oocytes incubated with spermatozoa from boars of low fertility showed decreased ZP-binding ability. Results obtained from a homologous in vitro penetration test revealed that both penetration rates and mean numbers of spermatozoa per oocyte differed depending on the fertility group of the boar (low, intermediate or high) (Gadea et al., 1998). Although Berger et al. (1996) could not demonstrate a correlation between in vivo fertility and sperm-ZP-binding capacity, they did find that the ability of spermatozoa to fuse with the oolema of zona-free hamster oocytes was correlated to in vivo fertility.

3.3. Ovine and caprine

IVF tests has also been proposed as methods for assessing the semen of rams and goats. For assays in these species heterologous oocytes have frequently been used, especially in the goat, from which homologous oocytes are difficult to obtain in large numbers. Berger (1989) used zona-free hamster oocytes in an assay of goat sperm, and Cox et al. (1994) suggested that cattle and sheep oocytes can be used to assess the fertilizing ability of goat spermatozoa.

The effect of individual rams on the fertilization rate after IVF, as well as on early embryonic development was demonstrated by Fukui et al. (1988). Smith and Murray (1996) found differences in fertilizing ability not only between individual rams, but also between ejaculates from the same ram. Attempts to correlate test results and in vivo
fertility using ram semen have given contradictory results. Choudhry et al. (1995) found
a correlation between the number of penetrated sperms per zona-free hamster oocyte and
in vivo fertility while Codde and Berger (1995) were unable to demonstrate a correlation
between in vivo fertility and the ability of spermatozoa to bind to or penetrate ZP,
although there were significant differences between rams among these in vitro parame-
ters.

3.4. Equine

Equine oocytes to be used in assays for evaluation of stallion semen have been
obtained from ovaries of slaughtered or live mares (Fazeli et al., 1995b; Pantke et al.,
1995). The oocytes have been used in ZP-binding assays, as intact ZP or hemi-zonas.
Further, stallion semen has been assessed for fertilizing ability in an assay using
zona-free hamster oocytes (Brackett et al., 1982b).

Semen from subfertile and fertile stallions was assessed in the ZBAs, and the results
were related to in vivo fertility (Pantke et al., 1995; Meyers et al., 1996). In these
studies, the in vitro fertility, expressed as the total number of spermatozoa bound to the
ZP or as a sperm binding index, was better in fertile than in subfertile males. Fazeli et al.
(1995b) developed a hemi-zona assay for stallion semen, and they found a significant
relationship between the number of spermatozoa bound and fertility of the stallions.

3.5. Canine

In the canine species, ZP-binding and penetration tests have been used to evaluate the
effect of different sperm treatment methods, such as cooling or freezing, on the
fertilizing ability of the spermatozoa (Hay et al. 1997b; Ström Holst et al., 2000b). In
addition, a HZA for testing dog semen has been developed (Mayenco-Aguirre and Pérez
Cortés, 1998). Since only small numbers of oocytes can be obtained for the tests via
ovariohysterectomy, the oocytes have to be stored before use. Ström Holst et al. (2000a)
demonstrated that the storage method used could effect the binding of the spermatozoa
to the ZP.

4. Conclusions

Fertility results obtained in an assay of a sperm sample are, strictly speaking, only
applicable to the sample analysed. In view of the inherent variation among ejaculates
and the tendency for male fertility to vary with time, it seems unlikely that male fertility
could be accurately predicted using sporadic in vitro tests. Longitudinal studies in which
both in vivo and in vitro fertility are periodically monitored are therefore necessary.
Further, fertilization is a very complicated process to be able to be completely tested
during the in vitro conditions we have at our disposal at present. We still do not have
any reliable method that mimic the complicated and important interactions between the
spermatozoa and the female genital tract that occur during the transport of spermatozoa
to the site of fertilization. Nevertheless, the different assays based on IVF techniques
seem to represent methods able to test the fertilizing ability of the spermatozoa. Used singly or in combination with other tests, they will make it possible to screen prospective semen donors, as well as to assess insemination doses or methods of semen preservation. Although these techniques cannot be substituted for in vivo testing in species like the bovine, they can be used to make a first screening of the young bulls, thereby allowing semen with a lower fertility to be excluded from test inseminations.

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References


