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Artificial insemination in pigs: Possibilities of future improvement in reproductive performance

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Artificial insemination (AI) for pigs offers many advantages in different fields (health, management, genetic improvement, etc.) which are clearly greater than the ones found in natural breeding. Problems with technical transfer and the traditional mentality of pig farmers and some established economic interests, however, delayed the development of this technique.

AI in pigs was slow to develop in the past, but in recent years its use has increased in importance because of technical improvements, the establishment of both public and private artificial insemination centers, and changes in farmers' mentality.

From a partial point of view, pigs producers have been very demanding as far as productive reproduction results are concerned, taking as the main productivity parameter the number of piglets/sow/year. They give less importance to offspring productivity controls as feed conversion, gain speed and carcass quality, sometimes refusing AI fearing a decrease in their production of piglets.

Current pig meat charges control are compelling farmers into a more careful balance of feed conversion and carcass quality, together with the number of piglets/sow/year, and this obliges them to take in consideration the breeding animals and their transmissive characteristics.

This evolution of farmers' mentality is increasing the utilisation of AI in pigs. The number of Spanish pig producers who have become AI users has considerably increased and they are the main promoters of this technique.

I - Current utilisation of AI in pigs

At the first International Conference on Deep Freezing Semen, which took place in Uppsala, Sweden, in August 1985, Dr. Reed presented very interesting data showing the use of fresh and frozen semen from 19 countries (**Table 1**).

In this table we can observe that the development of AI with fresh semen is very large in some countries, although a great number of them are still under 100,000-150,000 first inseminations, which represent a low average compared to natural mating.

On the other hand, a very low number of first inseminations with frozen semen is reported. That means that the technique must be improved in order to get better fertility results to increase the credit among pig farmers and veterinarians involved in pig production.

Inseminations with frozen semen have been done mainly in research centres and commercial units interested in semen distribution, notably by companies from the USA, where such techniques have developed faster than in Europe.

II - Semen contrastation techniques

Semen control greatly affects AI results, especially in Mediterranean countries where seasonal temperature changes are very strong and where summer temperatures are normally high enough to influence boar reproductive characteristics.

Semen contrastation techniques in boars are essentially similar to the ones practiced on other mammals. Initially, motility, track motility and acrosomal integrity can be considered as the most classical. Among them, motility is the simplest and most practical one, although it does not show a good correlation with fertility according to several authors.

The competitive index computed from fertility data has been highly correlated with many semen quality analyses that are frequently used. O'Connor *et al.* (1981) correlated visual motility estimates, computer determinations of motility and velocity, track motility and acrosomal integrity with fertility (66-day non-return rates) and the competitive fertility index. There were no significant correlations between fertility and laboratory analysis. However, there were significant correlations between the competitive fertility index and many of the laboratory analyses. The highest correlation (r = 0.93) was the fertility index with track motility.

Lysikiewicz and Enhorning (1983) introduced a tri-color exposure method for measuring track, motility and velocity. The film was initially exposed with green light followed immediately by brief exposures using red and blue light. Immotile cells appeared white, while motile cells showed a green track followed by a red and then blue image. Velocity was determined by measuring green track length.

The determination of acrosomal integrity is also one of the most practical semen quality assays and although it is not a definitive assay in correlation with fertility, it is used by most of the authors as one of the best controls.

Recently, Premzl (1985) presented fertility results from frozen boar semen classified by the average of normal acrosomes, with increasing fertility results in correlation with the increasing number of normal acrosomes.

Acrosome morphology and motility or sperm survival post-thaw are used to evaluate freeze-damaged spermatozoa. Korban *et al.* (1983) reported significant correlation between frozen boar semen fertility with percentage of damaged acrosomes (r = 0.84) and sperm survival time (r= 0.64).

The Sephadex filter test, thermo-resistance, cold shock or osmotic shock are also useful tests to be used in practical conditions.

Weidel and Graham (1984) reported that the Sephadex filter removes more cells with damaged acrosomes than other morphologically abnormal cells.

Recently, an *in vitro* assay for evaluating fertilizing capacity has been developed. This sperm penetration assay (SPA) tests the ability of spermatozoa to penetrate zona-free hamster ova. The SPA has been widely used for evaluation of human spermatozoa where *in vivo* fertility evaluation is difficult.

SPA has also been applied to goat semen (Kim *et al.*, 1980), boar semen (Majerciak *et al.*, 1982), bull semen (Wright, 1982; Baird, 1983) and guinea pig, and mice semen (Binor *et al.*, 1982). Blzak *et al.* (1982) combined SPA with heterosperic fertilization to develop a competitive *in vitro* fertilization assay for human semen. Sperm from different males were labelled with different colour fluorescent markers.

Many researchers are applying chemical and biochemical analysis for semen quality evaluation. Kantsedal (1982) reported that fertility with frozen bull sperm increased as the sodium/potassium ratio in semen increased.

Measurements of sperm associated enzymes and high energy phosphates are used to evaluate damage during freezing. Strzezek *et al.* (1979 and 1983) reported significant increases in LDH (lactic dehydrogenase), GOT (glutamic oxalacetic transaminase, aspartic amino transferase) and acrosin, inhibition of ATP (adenosine triphosphate) synthesis and a drop in the respiration coefficient in boar, bull and ram semen with sperm processing techniques.

Korban *et al.* (1979) reported that LDH, acrosin and hyaluronidase were more directly related to fertility of frozen semen than sperm survival and motility. Moroz *et al.* (1982) reported significant correlations between the fertilizing ability of frozen boar semen with hyaluronidase, acrosin and LDH activity.

Hyaluronidase and acrosin are enzymes used to evaluate acrosome integrity. LDH, GOT and GTP are enzymes released to the extracellular media when the membrane surrounding the midpiece and tail are damaged. LDH has been specifically identified as a midpiece associated enzyme. ATP synthesis specifically evaluates the integrity of the mitochondria in the midpiece.

Velocity measurement may also indirectly evaluate mitochondrial damage because sperm velocity increases as ATP concentrations increase.

In recent years both transmission electron microscopy (TEM) and scanning electron microscopy (SEM) studies have been used to evaluate semen morphology and damage.

Although these techniques can detect and locate damage within the sperm cell not seen by other techniques, they are time consuming and costly. It is not presently feasible to use electron microscopy for routine analysis of samples but its research application can be extremely valuable.

III - Semen storage

Stored semen is understood to be semen which has been prepared under such conditions that it can be used at least one day after collection for reliable insemination. It is different from fresh ejaculate which is used both diluted and undiluted immediately after collection, and where a temperature of 37°C is mantained until its application.

Fresh semen application is widely used in some centers where an insemination unit is available. Thus, each boar has a specific collecting day. Every morning the needful doses are prepared relating to the number of sows in oestrus. A second insemination is done with semen from the same boars or with sperm from other males collected the next day.

Inseminations with undiluted fresh semen are seldom used, just being practiced sporadically when few (2-3) sows are available for insemination.

Semen storage can be done by refrigeration or freezing. Lyophilization (freeze drying) is a storage system not successfully developed.

Cooled semen

Refrigeration temperatures in cooled semen storage can be 5°C, needing in this case cryoprotectors (Kato and Kasuya, containing egg yolk, are appropriate mediums), and 15°C, which are the most widely used temperatures around the world.

For practical cooled semen utilization we have established two preparation techniques, depending on the storage period (**Tables 2 and 3**):

a) Short storage periods (1-3 days)

– Semi-complete ejaculate collection (greater use of the semen)

– Dose preparation (70-100 c.c.) in BL1 or Kiew diluent to reach a spermatozoa concentration of 3 x 10^9 spz/dose

– Refrigeration between 15° and 18°C (constant temperature with an oscillation rage lower than 2°C)

– Maximum storage period: three days.

b) Long storage periods (1-5 days)

– Selection of boars presenting a good individual semen storage capacity and spermatic quality

– Ejaculate rich fraction collection (60-100 c.c.)

– Collecting frequency:
one collection/week to young boars
one or two collections/week to adult boars

– semen dilution 1:10 with MR-A, SCK-7, Zorlesco, Modena, BTS

– Cooling at 15°-18°C

– Diluent addition to 70-100 c.c./dose before insemination.

Intermediate storage periods can be achieved by using Kiew and BL-1 diluents and preparing the sperm in the same way as indicated for long storage periods.

When long storage diluents are employed on semen to be used for insemination one to three days after, the sperm remains very stable and shows a very high quality, increasing by the way prolificacy results.

Johnson and Aalbers (1984) have reported the results from a comparative study (**Figure 4**) where BTS, Modena and Kiew diluents are tested, with BTS the one which presents the most valuable results. After this experience, born out by other research teams, BTS has become one of the most widely used diluents in cooled semen storage, replacing Kiew medium.

The diluent used most in Spain for six to seven day storage period is MR-A. Storage results in comparison with other diluents have been very satisfactory, with MR-A showing a fertility rate of 76;10% and a prolificacy of 8;8 piglets at birth after an eight day storage period, being both higher than the other diluents in the same conditions (Perez Lys *et al.* 1984, **Table 5**), (Martin Rillo *et al.*, 1984, **Table 6**).

The addition of antibiotics to the boar semen storage medium is a common practice although they must be carefully selected according to the bacterial floral sensitivity against them.

From our own experience (Martin Rillo *et al.*, 1984) a maximum of sensitivity is achieved by use of Amikacin, Dibekacin and Gentamicin (**Table 7**). Good growth inhibition control can be achieved by a joint application of these antibiotics. On the other hand, no significant effect of Penicillin and Streptomycin has been observed in this experience, results corroborated by Dr. Senegacknik in another experiment.

High contamination rates due to *Staphilococcus aureus* and *epidermidis*, *Streptococcus piogenes*, *E. coli* and *B. hemotyticus* have been found in boars, which decrease the storage capacity and fertility results in the semen from those animals. The addition of 200 mg of Gentamycin and 200 mg of Polimyxin B/1 of diluent achieves contamination control and subsequently improves the fertility rates.

Frozen semen

After the first frozen boar semen fertility results at the beginning of the 1970s, many research teams worked in this field until 1973-1974: Polge *et al.* in the United Kingdom, Graham, Pursel and Johnson in the USA, Paquignon and Courot in France, Crabo, Einarson and Larsson in Sweden, Sarmiento and Vazquez in Spain, Troeu and Westendorf in West Germany, and Senefacnik in Yugoslavia.

Semen freezing techniques are normally based on the following steps:

Initial equilibration: the seminal plasma contains lipoproteins which are profitable in the cell membrane protection. In some techniques, an equilibration of the rich fraction not diluted or diluted just with saline serum is recommended.

Centrifugation: to eliminate salts from the seminal plasma.

Semen dilution: spermatozoa concentration about 600,000 spz/mm or lower are considered the optimum by some authors.

Diluents: egg yolk with Tes or Tris and sugars, with BF5 being used most (Pursel *et al.*, 1975) and the one containing just sugars as glucose or lactose. OEP addition (Sodium lauryl sulphate) (Graham *et al.*, 1971) has been generally accepted, and butylhydroxtoluol, an antioxidant proposed by Kononov, also improves freezing efficiency. Glycerol addition to the diluent before freezing at 1-2% is widely used.

Equilibration time: an equilibration period of two hours is normally recommended, although Pursel (1985) proposes a longer one when the medium contains just sugars.

Cooling speed: it must be slow until a complete cell protection and then semi-fast by use of nitrogen vapours or dry ice.

Freezing geometry: pellets on dry ice, macro-straw (5-8 ml) or micro-straws (0.5 ml), proposed by Vicente Sarmiento (1970), have recently been greatly developed by German (Weitze *et al.*, 1985) and Yugoslav researchers (Premzl *et al.*, 1985).

Thawing: straws in water bath depending on the time and temperature of the straw size. For pellets, thawing on a hot plate or diluted in BTS,

OLEP or INRA-ITP diluent, is recommended (Paquignon *et al.*, 1985).

Results achieved with semen do not allow, at present, cold semen insemination substitution in AI centers although, as is shown in **Tables 8** and **9**, those results are being each time with more and more promise.

The individual boars semen freezing capacity is fundamental and thus, some boar semen would present difficulties in freezing whilst others could be efficiently used.

IV - Refrigerated dialyzed semen

In order to enable long distance transport of refrigerated semen, we collaborated in our Institute with Dr. Graham and Dr. Pursel in common research. Several semen concentrations were studied. The dialysis joint with a long storage diluent was the method which has shown the best results at the laboratory level.

Two fields trials were done in order to verify the reliability of this technique. One of them was in a pig farm located 500 km away from our Institute and the other one between Spain and the USA.

The preparation method, which is shown in **Table 10** is:

1. Spermatic fraction collection
2. Dialysis tube filling (pore = 2nm) according to Vazquez and Graham technique (1980)
3. Dialysis against MR-A medium (1:10) 37°C for 30 minutes using a magnetic stirrer and descending temperature to ambient.
4. Racking to a container and transportation at 15°C.
5. Dilution 1:5 and storage at 15°C until utilization.

Fertility rate and prolificacy number results (seven days storage) were 84.6 and 8.54 respectively, both in Spain and USA (transportation). This experience was made on purebred Duroc sows, where prolificacy is lower than that found in production hybrid sows.

This technique appears as one of the best to be used in refrigerated semen with transportation because of its results and the short volume occupancy as well as the facility to control the transport temperature by the use of an acetic acid block in a polyspan box.

The complete results from this experience are shown in **Table 11**.

V - Further possibilities in the reproduction field by AI

Because artificial insemination is often used for genetic improvement, the most important problems are the ones affecting semen storage, which has not benefited from a complete development of artificial insemination in swine.

Lately, research on boar semen freezing has been increasing, and thus the causes of difficulties in freezing are being discovered. In the very near future a high percentage of boar semen will be frozen and used in AI competitively with fresh and cooled sperm with regard to fertility results.

Artificial insemination in pigs has a very promising and relatively undeveloped research field, which is fertility and prolificacy increase by means of the introduction into the female genital tract of other elements beside the spermatozoa.

In this way, many advantages can be achieved:

Improving metabolism and cell stimulus, enhancing both the movement patterns and speed of the spermatozoa with a subsequent augmentation of fecundating rates. PGF2α addition, recently studied by several researchers, results in fertility increases, as has been demonstrated by Tekacs *et al.* (1985) although the addition of 5 mg of PGF2α shows a shortening of the spermatozoa movement quality *in vivo*. The positive correlation of low ATP content in frozen semen with low fertility, as has been shown by Albers *et al.* (1981), is a research line to be investigated in the future.

Another way to increase fecundity probabilities is the augmentation of the spermatozoa population in the oviduct by means of a treatment to stimulate female genital tract contractions. Both oxitocin and carbetocin have shown good stimulation results according to several authors (Kozumplik *et al.*, 1984, and Riechel *et al.*, 1984) although their work is discussed by other research teams. Researchers such as Rodriguez Martinez

and Einarsson (1985) advocate the use of prostaglandins to activate the oviduct motility, and some authors have indicated other elements such as carbacol and several rye ergot derivatives as sow genital tract contraction stimulants.

The addition of some substances for endometric regeneration and contamination control, such as some antibiotics and other drugs like Asiatic flake (*Centella Asiatica*) extract, can help the nidification and are probably able to decrease embryonic mortality.

Another pig production area to be investigated is reproduction immunology. Good knowledge of histocompatibility and sensibilization of the ovocytes, embryos and genital tract can be used to improve fecundation and nidification as well as to decrease embryonic mortality. This can be decisive in a significant increase of fertility and prolificacy results (Almlid *et al.*, 1981; Murray *et al.*, 1981; Blichfeldt *et al.*, 1984; Warren *et al.*, 1980; and Meziou *et al.*, 1983). Much research is now being done in this field but no practical results have yet appeared.

Currently, only physico-chemical controls are being applied to boar semen diluent mediums both for cooling and freezing processes. Further controls must be designed in order to increase fecundation capacity, spermatic transportation improvement, contamination reduction and promote optimal endometric regeneration and immunologic sensibilization, all directed to improving nidification and reducing embryonic mortality.

All these factors, combined with the perfection of dose application techniques, can achieve significant increases in the number of doses per ejaculate. At present, a 2 to 3 $\times$ 10^9 spz/dose is being applied for sow insemination, a population which must be drastically reduced in a very near future.

On the other hand, the great development of new reproduction advances, such as sperm micro-injection, *in vitro* fertilization, chimeras, gonogenesis, androgenesis, animal homozygous production, ova maduration, nuclear transplantation, animal trangenic, etc., can be the basis for further evolution of animal reproduction. Studies on semen and artificial insemination will not be a target of reproduction research, but they must be some very well known techniques to be used in the near future for new pig production investigations.

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