New advances in AI technology and application

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At this present time it is difficult to estimate the number of sows served with AI as a percentage of all services. Back in 1998 we were quoting a level of 35-40%, with Europe achieving 60-90% of their services via AI.

Since then we have seen a continuous growth in the sale of AI, and this has been against the background of a declining national herd, to such an extent that I would confidently claim that the UK is now on a par with AI usage in Europe.

The emergence of PMWS has also increased the conversion of units to batch weaning, which by its nature has also contributed to the growth of AI.

The benefits of using the superior genetics available through the use of AI are now well documented, with a reduced production cost of 5-7p per kg regularly being achieved.

However, there is still an inconsistency in the reproductive performance from the use of AI across the pig sector. The reasons for this are unclear and need to be investigated in more detail, but in the meantime we must endeavour to develop AI production protocols and insemination strategies that will facilitate the successful use of AI throughout the industry.

Therefore, this article will look at the optimum number of sperm required for a successful insemination, new insemination techniques and, finally, the development of semen additives to facilitate an effective insemination.

Measuring sperm concentration

Standard laboratory measurement of sperm concentration per ml of raw ejaculate is through the use of a colorimeter/photometer.

The instrument has been calibrated using at least 40 ejaculates to provide a range of readings and ejaculate characteristics so that a regression line can be calculated. An ejaculate's sperm content is then calculated by measuring the opacity of the liquid and relating this reading to the calibration chart, to give the sperm concentration per ml.

However, due to the variation found between boar ejaculates in the opacity of the seminal plasma, this can underestimate or overestimate the number of sperm by between one and 30%. The only direct method of counting sperm, until recently, was through the use of a counting chamber or haemocytometer, which is used to calibrate the colorimeter. However, this is a time consuming method and impractical for use on the processing bench.

New counting techniques

The development of a new counting machine in Denmark, the SP-100, has been judged to be almost as accurate as a flow cytometer, the 'gold standard' for sperm counting. It works by killing the sperm, staining the nuclei, fluorescing and then counting the stained nuclei of a known volume of ejaculate.

It is also extremely accurate in measuring sperm concentration in the final doses, more so when compared to the results obtained when using a haemocytometer, due probably to the infinitesimally small aliquot of diluted semen used by the haemocytometer method.

So, what is the optimum number of sperm per dose of semen? Unfortunately, there is no definitive answer. However, there are indications of the range of sperm dose that will give acceptable results, as demonstrated by the Institute of Pig Genetics in the Netherlands.

Their findings were confirmed in Denmark, by the National Committee for Pig Production, during a trial comparing DIY-AI and AI purchased from the Danbred AI centres.

In general, we can conclude that acceptable results are obtained from inseminations containing between one and eight billion sperm per dose.

Deep insemination

The advent of deep AI over the last 12 months has raised some interesting questions and possibilities. The principle is to deposit the semen through the cervix and into the uterine body.

The advantages are:

- Little or no leakage is observed with deep AI.
- No sperm are lost in the mucous and cervical folds of the cervix.
- Less time is required for the introduction of the semen.

The disadvantages are:

- Inseminators have to be trained, patient and confident.
- Variable time required before the introduction of the insert.
- Gilts can only be served with the Absolute 'Membrane' Deep AI catheter.
- This plastic insert technique cannot be used on a small percentage of sows.

Is backflow during insemination contributory to a poorer reproductive performance? Work from the Netherlands would indicate that it could be implicated in reduced fertilisation rates in situations where up to half the inseminate was lost during insemination.

This effect was more pronounced towards the lower range of sperm per dose and so any technique that can reduce the incidence of leakage will likely be beneficial.

On farm trials with the deep AI technique are being conducted at this moment in the UK and initial results look promising, reflecting those already obtained in Europe.

The immune response

The review of the trial data from the Netherlands and Denmark suggests that sperm dose per se is not an indicator of reproductive performance and the apparent improvement in performance with deep AI would indicate that there must be some other mechanisms that are influencing the final outcome for the sperm.

These will probably include the interaction between the efficiency of sperm transport and the immune response to the presence of the inseminate. It will, therefore, be useful to review what happens to the sperm once it has been inseminated.

Within minutes of the insemination being completed, the first sperm are reaching the sperm reservoir at the end of the oviduct adjacent to the utero-tubal junction.

In response to the insemination the uterine lumen is massively invaded by polymorphonuclear leucocytes (PMNs) approximately 30 minutes post insemi-

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nation. Uterine distension, seminal plasma and sperm presence have all been suggested as mediators for this PMN recruitment. Those sperm not already voided by retrograde reflux at this time are then removed by phagocytosis by the leucocytes over a period of hours.

There appears to be a 'dose dependant' response in that the greater the number of sperm, the greater the number of PMNs that are recruited.

Benefits of low semen dose

Conversely, a lower concentration of sperm appears to reduce the level of phagocytosis of the sperm. Inseminating a lower volume of sperm also reduces the amount of semen lost through backflow. So the reduction of sperm dose is not mirrored in the reduction of the sperm number at the site of fertilisation.

However, because the convoluted structure of the uterus is essentially a hostile environment to the sperm we must try and encourage the fast movement of the sperm through it to the oviduct, where it is stored safely until required.

Using the deep AI technique is already giving the sperm a good start and from the point of deposition at the head of the uterine body, the transport of the sperm is primarily a function of uterine contractions.

When a sow is in oestrus, the frequency of these contractions for sows with spontaneous uterine activity has increased from less than 10 per hour to over 20 per hour on days one and two of oestrus.

However, there is a large variation between the sows exhibiting this spontaneous uterine activity.

One method by which these sows with a low level of spontaneous activity can be stimulated into an increased frequency of uterine contractions is through the presence of a boar, which stimulates the release of oxytocin.

Semen additives

Any method which can be developed to manipulate the leucocytes influx and resultant phagocytosis of the sperm and encourage sperm transport to the oviduct is, therefore, likely to increase the numbers of sperm available at the site of fertilisation and hence the fertilisation rate.

One obvious route to influence these physiological responses would be through the use of semen additives and so the rest of this article will look at two potential additives and a third that is a 'sperm activator'.

The use of deep AI has opened up the possibilities of introducing a post-AI flush without extending the insemination time by any appreciable amount.

It will, therefore, be interesting to introduce substances in this post-AI flush that will help modulate the leucocytes influx and phagocytosis and also help stimulate uterine contractions.

There are two products that will be available shortly:

- Oxytocin analogue.
- Immunosuppressant.

Oxytocin analogue

There has been a considerable amount of trial work using oxytocin with AI.

This has been either in the form of injecting the sow at the point of insemination or introducing oxytocin into the semen dose just prior to use.

The latter is now the preferred route of introduction. The results have been ambiguous but general conclusions are:

- It is worthwhile including oxytocin to improve litter size or farrowing rate where reproductive performance is reduced.
- Use of oxytocin is more beneficial in sows than in gilts.
- During the summer months the use of oxytocin significantly increases farrowing rate and litter size.

One of the basic requirements for a good insemination is for the sow to be stimulated by the boar. Unfortunately, this is often the area which fails in many service facilities.

The majority of the boars become disinterested after 15-30 minutes and go to sleep. Keeping them in pairs can help, but the reality is that boar 'presence' is not enough and so stimulation is minimal.

Once the AI has been completed sows are often removed from the insemination pen and introduced into a resting area with the remaining sows from her group.

This pen usually supplies boar contact but not always and she is also open to negative interactions from her peers that can interfere with the sperm transport.

Addition of the oxytocin analogue can then be regarded as insurance against any environmental effects that can be suppressing the sow's potential level of myometrial contractions.

Immunosuppressants

The previous research work using an immunosuppressant has shown an encouraging reduction in the level of polymorphonuclear leucocyte activity leading to an improved sperm number in the oviduct.

This increased sperm reservoir then provides sufficient sperm at the site of fertilisation over a greater period.

The number of sperm present is measured by an accessory sperm count that shows how many sperm meet the ova post-fertilisation and become attached to its outer layer.

Sperm activator

Motility is a common method by which an ejaculate is assessed for its potential fertility.

Unfortunately, the correlation with resultant fertility is low.

Assessing motility will, however, identify overtly poor ejaculates, provided that the assessment is performed correctly.

This lack of correct procedures for stimulating the sperm to express their motility is a major problem in the industry at the moment.

A protocol sanctioned by the breeding companies has been developed that highlights what inputs are necessary to achieve a representative motility score. The essential input is incubation time and temperature of the semen.

With the widespread use of long life diluents this becomes even more critical as the visual motility from these type of diluents becomes depressed.

Some samples have to be incubated at 38°C for up to one hour before they have expressed their motility.

However, Bio-Innovations, a JSR Genetics subsidiary, has developed a sperm activator that works almost instantaneously on semen direct from the temperature controlled cabinet at 17°C.

This removes the need for correct incubation conditions and gives an instant answer as to the true potential for the number of sperm able to become motile. It is to be hoped that this will become the standard method for assessment in the future.

Conclusion

In the UK the performance from using AI as the main service type has yielded variable results.

Some units achieve consistently good performance, whilst others continue to under achieve. The reasons for this are complex and the causal factors for this variation are difficult to identify.

Applying new technologies to help counter any negative environmental effects will hopefully prove beneficial in reducing this variation, allowing more producers to achieve a more consistent level of production. ■