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Artificial insemination in pigs, research and developments in The Netherlands, a review

Inseminação artificial em suínos, pesquisa e desenvolvimento nos Países Baixos, uma revisão

Hanneke Feitsma

INTRODUCTION

The fertility results of pig artificial insemination (AI) in The Netherlands are considered to be one of the highest worldwide. More than 98% of the sows in The Netherlands is mated through AI. Sow farmers put great trust in AI and are fully depending on the quality of the product. The Dutch cooperative pig AI centers put large efforts in producing high quality insemination doses as far as semen quality and genetic quality.

In this paper the following aspects of AI will be discussed: History of Dutch pig AI, success factors of Dutch pig AI, status of pig AI in the Netherlands, semen production at Dutch pig AI centres and Dutch pig AI research and development results. The emphasis will be put on: effects of semen motility, sperm number and semen age on the fertility results and sources of variation in semen quality. Furthermore a vision of future research and developments will be given in the last paragraph.

HISTORY OF DUTCH PIG AI

At the end of the 60-ties in the past century, pig AI became available for pig breeders in The Netherlands. The use of pig AI was highly stimulated by the Dutch Animal Health Services and together with the public body for agriculture, large emphasis was put on the development of pig AI. Main reason was to prevent the spread of contagious diseases, since it was common practice that breeding boars were transported from one breeding farm to another breeding farm to mate sows. The first pig AI-stations were funded as independent cooperative boar stations and populated with first choice breeding boars. The AI companies and breeding companies were working closely together in these projects to make the genetically highest indexed boars available for AI.

In the beginning of the use of pig AI results were poor. Pregnancy rates of 60% and litter sizes of five to six piglets were common. However the Dutch pig industry was convinced of the advantages of AI and invested time and money in further research and development. In the years 1980-1990 the results improved tremendously due to the knowledge gained on: the oestric cycle of the sow, the timing of the insemination and the proper treatment and dilution of boar semen. The AI results levelled with the results of natural mating (NM) and AI became advantageous to use at commercial farms as well. This was the period in which the use of pig AI increased rapidly.

Until that moment inseminations were performed by specially trained AI technicians, but from the moment commercial farms were involved, the demand for self service AI was born and increased together with increased demand for commercial semen.

Once the Dutch pig AI companies started to use Beltsville Thawing Solution (BTS), developed by the USDA laboratory in Beltsville, freshly prepared semen could be stored for up to 48 hours instead the 24 hours until than. Since the results of cryopreserved boar semen were significantly lower than with fresh semen and the logistic infrastructure in The Netherlands is arranged very efficient, Dutch farmers preferred to use fresh diluted semen.

Institute for Pig Genetics B.V., Beuningen, Gelderland/ The Netherlands. CORRESPONDENCE: H. Feitsma [hanneke.feitsma@ipg.nl].

SUCESS FACTORS DUTCH PIG AI

Success of pig AI use in The Netherlands is based on six factors:

1. Health: using AI diminished the risk of disease introduction in a herd.
2. Breeding efficiency: AI enabled the breeding companies to select the best indexed boars and use these boars more intense in the nucleus breeding than in the case of NM. Hence their genes would be spread more effectively and the genetic improvement in a population would increase more rapidly.
3. Economics: Once the results of AI levelled up with the results of NM, AI was cheaper than NM. Farmers did not longer need boars to mate the sows and could easily gain some extra money by replacing the boars by sows and saving time in the mating pen.
4. Collaboration: Strong collaboration between the pig AI companies and the pig breeding companies made it possible that only the best boars would come into the boar stud. The pig AI centres still have the first choice in the young boars produced. On the other hand AI companies were dependant on the quality of the breeding program of the breeding organisation and the quality of the boars. Therefore AI companies raised fees over their tariffs and used this as "stimulation money" for the breeding program of the breeding organisations.
5. Knowledge: The Dutch cooperative pig AI centres structurally invest in basic and applied research since 1977. Per doses sold, an equivalent of 0.05 per dose sold has been invested. The knowledge gained by research and the tight connection between researchers and cooperative AI companies enabled the AI companies to use the newest techniques and best practice.
6. Data recording and ICT: Data recording both on pig AI centre and nucleus herd level was used, first on paper. With the introduction of computers and central databases for breeding and AI, a standardised calculation of technical results and (electronic) data exchange, became available. Herewith large datasets became available for analysis. Linkage between breeding and AI datasets made it possible to retrospectively analyse the relation between semen quality characteristics and fertility results. For example, new techniques can be tested in an infrastructure which enables the Dutch pig AI companies to decide whether introduction of this new technique is valid. Therefore field trials are designed in a such way that fast, accurate and easy data retrieval is possible and differences in litter sizes as far as 0.1 piglet can be detected.

STATUS OF PIG AI IN THE NETHERLANDS

In Europe, the pig AI rate – i.e. percentage sows mated through artificial insemination – is between 25 and 98%. In The Netherlands the pig AI rate is 98%. About 97% of the insemination doses are purchased from AI companies of which 90% through the cooperative pig AI centres Varkens KI Nederland and Varkens KI Twenthe. Only 3% "on farm AI" is used. Boars located on the AI centres origin from several breeding companies such as TOPIGS (94.9%), PIC (3.4%), Nord West (0.6%), Danbred (0.3%), JSR (0.1%) and others (0.7%). Table 1 illustrates some characteristics of sow production and pig AI in The Netherlands.

Table 1. Characteristics of Dutch piglet production and AI between July 2007 and June 2008. Inseminations are performed with intra cervical catheters.

Nº sows:	1.000.000
Nº AI doses sold	4.200.000
Nº cycles per sow/year	2.35
Nº doses per sow per cycle	1.8
Minimum nº cells per dose (total)	$1.5x10^9$
Farrowing rate	86%
Total number born per litter	13.9
Live number born per litter	12.9
Nº piglets weaned/sow/year	26.2

In contrast with a lot of other countries, where inseminations are alternated with natural mating within a cycle of a sow, AI is solely used in The Netherlands. The Dutch were the first to introduce the pooling of semen (since 1992) for commercial herds, however since the major outbreak of Classical Swine Fever in 1997, where two pig AI stations were involved as well, the use of pooled semen was prohibited by the Dutch government. Pooled semen complicated the effective tracing of potentially infected boars and increased the risk of virus spread over farms in their opinion. Since that time, a few times per year, boars were detected with low farrowing rates and small litters. These boars were detected because of the data recording and monitoring of the boar performance in the field. Since no reduced sperm quality was detected, another reason had to be the cause. After thorough investigation, it was found that a so called Reciprocal Translocation (RT) – i.e. a type of chromosome rearrangement involving the exchange of chromosome segments between two chromosomes that do not belong to the same pair of chromosomes – was found in these boars. A monitoring project showed a prevalence of ± 0.7% in the population. Since that moment the breeding company TOPIGS tests all AI boars for RT. In spite of the low prevalence and the high test costs the testing is still in progress, since the negative effect of this abnormality is large. Furthermore, RT prevalence stays on the same level, since the anomaly arises rather spontaneous or is induced by medication, radiation, radicals etc. As a result of excluding these boars from the pig AI centres, the average litter size per first insemination increased with 0.05 piglet, an economic equivalent of 1.4 million. The test costs of maximum 200,000 justify the testing of all new AI boars before entering the AI station.

SEMEN PRODUCTION IN DUTCH PIG AI CENTRES

Semen production for commercial use in The Netherlands is only allowed on EU approved semen collection centres (Directive 90/429/EEC, European Union). Boars are introduced at AI centres via quarantine and strict health controls. The health status of AI centres is further guaranteed by strict bio security measures and health control programs amongst other annual serological control on each AI boar for Classical Swine Fever, Aujeszky's Disease and *Brucella suis*. From the seven AI centres connected to Varkens KI Nederland, three have the Specific Pathogen Free (SPF) status for Porcine Reproduction and Respiratory Syndrome (PRRS) virus, *Mycoplasma hyopneumoniae* (Mhyo) and *Actinobacillus pleuropneumoniae* (App) bacteria. Apart from disease control, boars from the TOPIGS breeding program are all tested for RT. In 1991 all Dutch AI centres suffered from a PRRS outbreak which had a tremendous effect on the semen quality and production [13]. Since that time internal calamity procedures are in place to deal with disease outbreaks at AI stations.

At all centres of Varkens KI Nederland, semen production is performed under strict hygienic control. A hygiene monitoring program is installed since 1994. In this program, critical points in the production are tested on a daily, weekly or monthly schedule for bacteriological contamination. In 2006, only six out of ± 2,000 samples were tested positive with one to two Colony Forming Units (CFU) per plate count agar. Environmental contamination of the plate was the probable cause. Boars are collected in separate collection pens on dummy sows or in an automated semen collection system (Collectis®, IMV). Before semen collection, body temperature is measured in order to ensure the health status of the boar on the collection day. The semen is transported from the collection area to the lab via a pneumatic tube system. At the lab, the semen is pre extended within 10 minutes after collection (Solusem®, equal temperature as the semen). Each ejaculate is assessed for semen quality [5,6,12]: macroscopic evaluation of colour, smell, contamination with dirt, blood, pus or urine and viscosity. The volume is determined, semen motility and concentration are measured in a Computer Assisted Semen Analysis (CASA) device, samples are taken for longevity testing and morphology analysis. After the quality evaluation the semen is diluted (Solusem®, 20°C) to a minimum level of 1.5 billion cells in 80ml and - via a full automatic filling device - put into tubes. Extender production is performed on the pig AI laboratories. Therefore, modern water production equipment is available. Water demineralisation is performed by reverse osmosis, water disinfection is executed by UV-lights. The quality of both production water and prepared extender is checked by measuring pH, conductivity, osmolarity and bacteriology. The insemination doses in tubes are transported from the laboratory to the distribution area. Laboratories as well as distribution areas are temperature conditioned.

For the transport of insemination doses to the clients, temperature conditioned cars are used. Transport temperature is 17°C. In The Netherlands almost all farmers have temperature controlled boxes, cabinets to store the doses until usage on the farm.

DUTCH PIG AI RESEARCH AND DEVELOPMENT RESULTS

Since 1977 the Dutch cooperative pig AI centres have special funding for research. From each insemination dose sold, 0.05 is spent on research. The overall research objective is to improve AI results at sow farms. Therefore research is performed in the areas of semen quality and relation to fertility [4,5,6,7,8,9], efficiency in semen production [5,6,11,12,14,16], bio security in semen production and improving knowledge on sow fertility [15]. In this way, not only fertility results through high quality must be ensured, but sound and efficient dissemination of genes of the breeding programs as well [15].

In the past, numerous PhD studies contributed to the knowledge of pig AI, fertilisation and gestation, financed by the cooperative pig AI companies and in collaboration with Universities and Research Institutes. The coordination of the research is organised in a way that the results of basic research are implemented in practice fairly quick after validation in field trials. The role of the Institute for Pig Genetics (IPG) in this process is to "translate" the results of basic research into practical implementations which serve the improvement objectives of the cooperative pig AI companies. IPG maintains databases of both breeding company TOPIGS (Pigbase) and the cooperative pig AI centres Varkens KI Nederland and Varkens KI Twenthe (KIS). PigBase contains over five million litters records whereas KIS contains the semen quality information of approximately 1 million ejaculates. By merging these datasets and link insemination doses to sow records, fertility results of these insemination doses and thus ejaculates can be analysed.

In the following paragraphs a few examples of analyses performed on these datasets – which improved the efficiency and quality of the AI - will be discussed.

Effects of morphological abnormal sperm cells on fertility results

The ejaculates of all AI boars are assessed for morphology at least each 6th ejaculate on routine basis. However when aberrations are observed or when otherwise the ejaculate quality is doubted, each ejaculate of that boar will be evaluated for longevity and morphology. Because morphological evaluations are laborious, the results are only available after distribution of the insemination doses. Therefore some ejaculates with higher abnormal morphology were used for inseminations. Due to the results of these ejaculates, it was possible to analyse the effect of abnormal sperm cells on the fertilisation results [2,7,8,9]. Table 2 shows the descriptive statistics of the dataset used for the statistical analysis. At that moment of analysis the dataset contained 588,931 ejaculates with data, of which 456,345 had no morphology data, and the remaining 132,586 ejaculates had scores. The ejaculates did not differ for volume. The higher the percentage abnormal sperm cells in ejaculates, the less ejaculates were sold. This indicated that, apart from morphological aberration, the microscopic evaluation of fresh semen already gave the lab technicians an indication of the reduced semen quality and therefore part of these ejaculates were rejected.

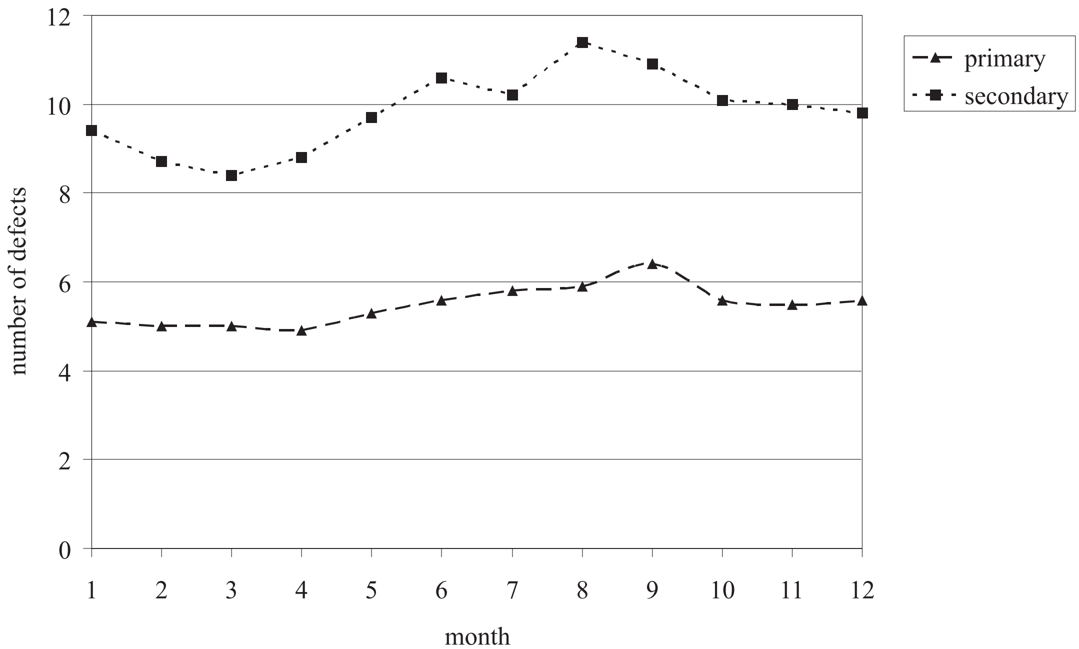
Table 2. Descriptive statistics of the AI dataset used for the analysis of the effect of morphological abnormal cells on fertility results.

Class	#	% Primary	% Secundary	% Sold	Ejaculate volume (ml)	Sperm cells/dose (10^9)
No morphology	456,345	-	-	90	263	2.82
Fine	41,032	2.0	4.2	81	267	2.85
Primary? 5%	27,875	11.5	4.7	71	268	3.01
Secundary? 10%	25,974	2.3	19.3	70	264	3.05
Poor	37,705	13.7	23.2	48	263	3.24
All with data	588,931	7.4	12.7	85	263	2.86

No morphology: no morphology assessment performed; Fine (primary defects ≤ 5%;secondary defects ≤10%);Primary: primary defects (abnormal head, acrosome and tail, loose head and bent tail); Secondary: secondary defects (proximal and distal cytoplasmic droplets); Poor primary defects ≥ 5%;secondary ≥ 10%)

Furthermore, one can see that increased percentage abnormal cells led to an increase in the average number of sperm cells available in an insemination dose, indicating that lab technicians compensated poor morphology by increasing the number of total cells. Whether this strategy works, could not be analysed in this dataset, since increased morphology and sperm number are confounded.

Morphology is affected by season. In Graph 1 the percentage primary and secondary defects per month, corrected for year and temperature effect (average from 1997 – 2006), is illustrated.



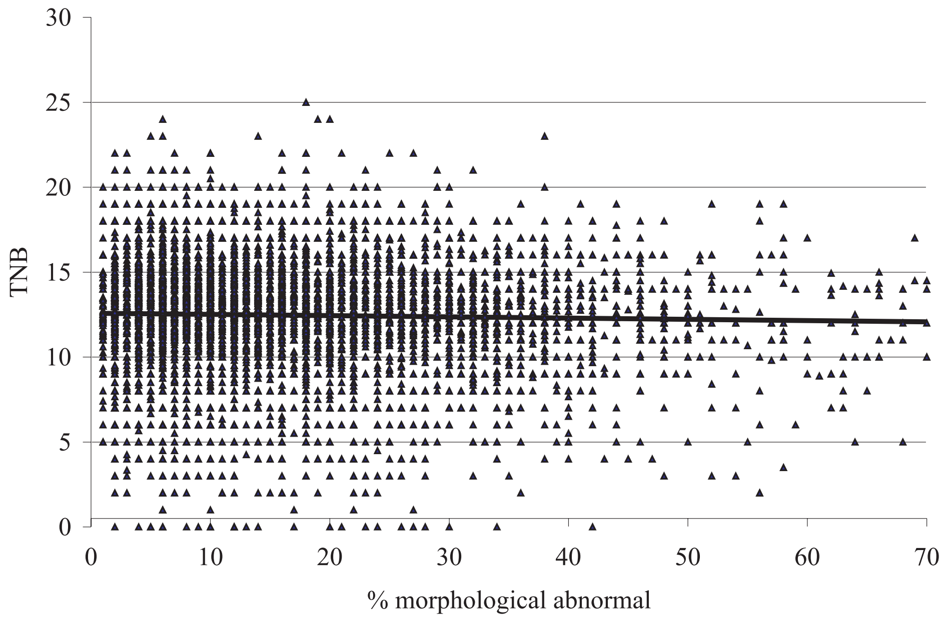
Graph 1. Percentage primary and secondary defects of boar ejaculates per month corrected for year and temperature.

Statistical analysis of the data showed an effect (Graph 2) of -0.3% for NR28, -0.7% for FR and -0.08 for TNB at an increase of the percentage abnormal cells in an ejaculate from 20 to 30%. The effects of percentage morphological abnormal cells on TNB are illustrated in Graph 2.

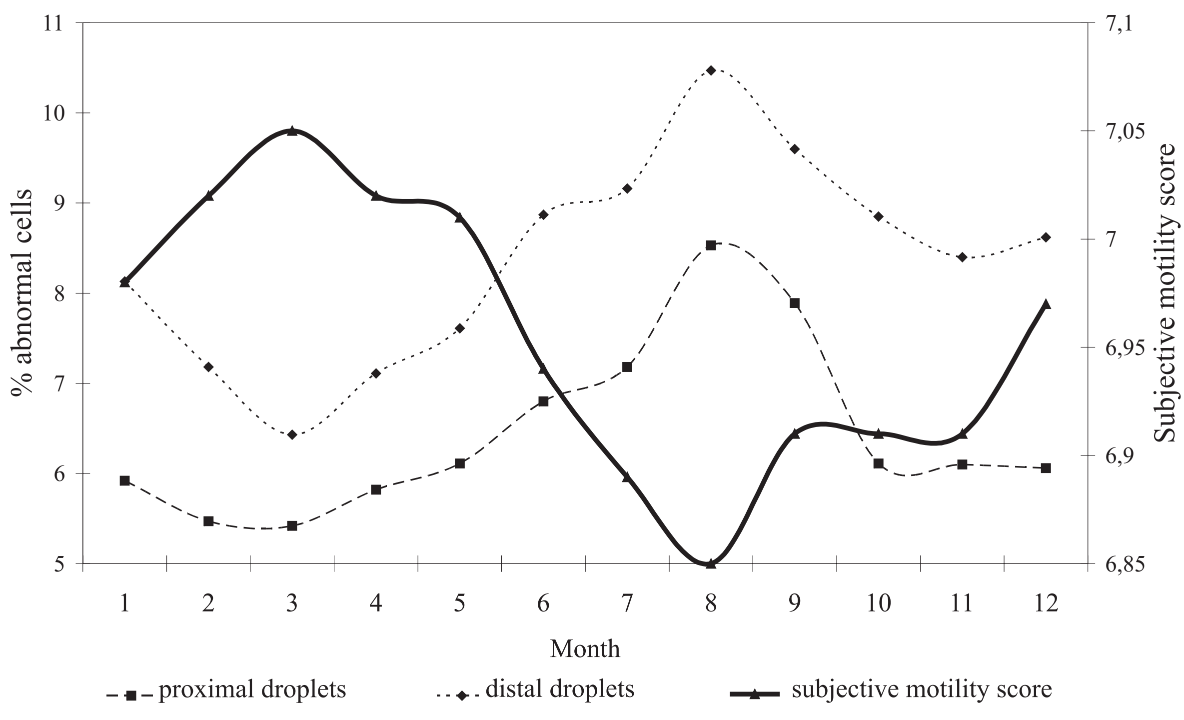
Although the effect for TNB is rather small, for the Dutch pig industry it represents four million euro. For pig AI centres it is therefore necessary to assess morphology before dispatch of semen. However in reality this is impossible because of the time the assessment takes and the relative short life span of semen and therefore the need for immediate transport to clients. Automated Semen Morphology Assessment (ASMA) therefore must be developed to overcome this problem.

Effects of semen motility on fertility results

Semen motility is been considered as an important characteristic for fertilising capacity of semen cells. Motile semen is assumed to be alive and intact whereas immotile semen cells are assumed to be damaged or dead. Semen motility has routinely been assessed using light microscopy with phase contrast with a 200 X magnification [5,6,12]. In the opinion of the Dutch cooperative pig AI centres however, the microscopic motility assessment is subjective and inaccurate. This was the reason why CASA-systems were installed in October 2006 at all AI laboratories [14,16]. Statistical analysis was performed retrospectively on the microscopic assessment data from 1996 until 2006 [4]. This analysis showed a significant relation between motility and fertility traits NR28, FR and TNB ($p=0.04$, $p<0.0001$ and $p=0.007$ respectively). Furthermore a significant relation between motility and morphology was found which is illustrated in Graph 3.

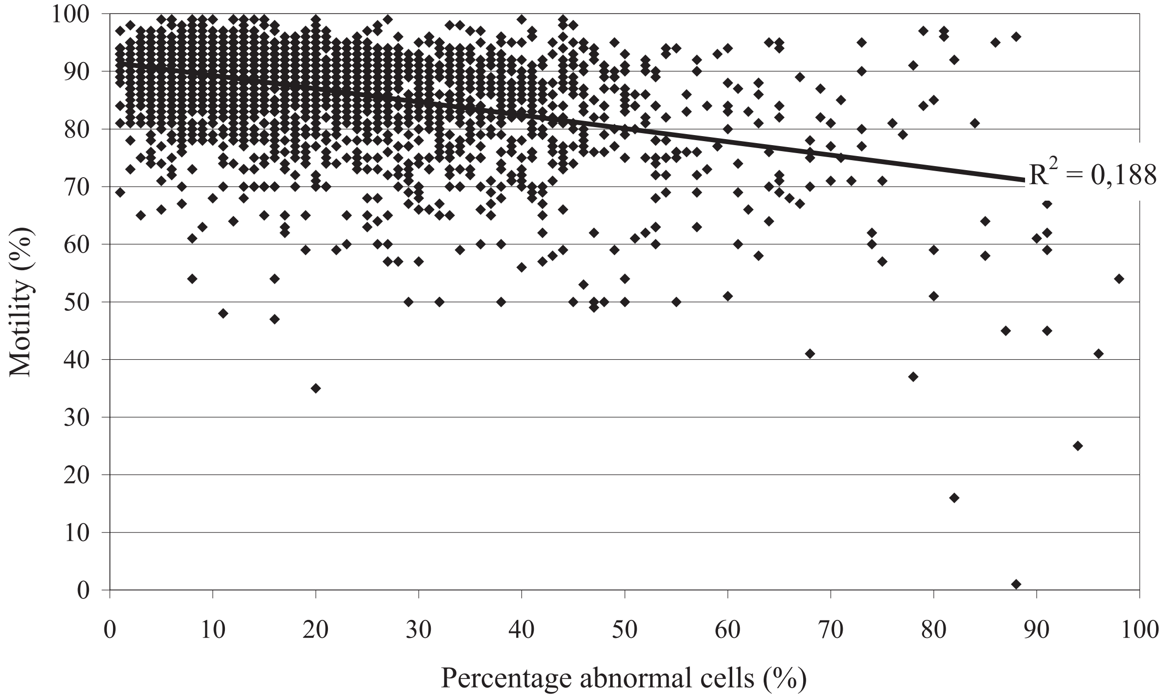


Graph 2. Relation morphological abnormal cells and total number of piglets born per litter.



Graph 3. Relation between percentage proximal and distal cytoplasmic droplets and microscopic motility score on a scale from 1-10 (10-100%)

Analysis of the CASA data of 2006 and 2007 is ongoing, however preliminary results show that the relation found between objective motility score and fertility is again and more strong compared to the microscopic motility assessment results. Besides the percentage of motile and progressive motile sperm, CASA systems score basic semen motility characteristics such as average path velocity (VAP), progressive velocity (VSL), track speed (VCL) and beat cross frequency (BCF). The challenge is to find combinations of those parameters which relate stronger to fertility and thus could be predictors for fertility. Remarkably, we found that the effect of morphological abnormal cells on motility as found in the subjective microscopic data is much smaller in the CASA motility data (Graph 4).



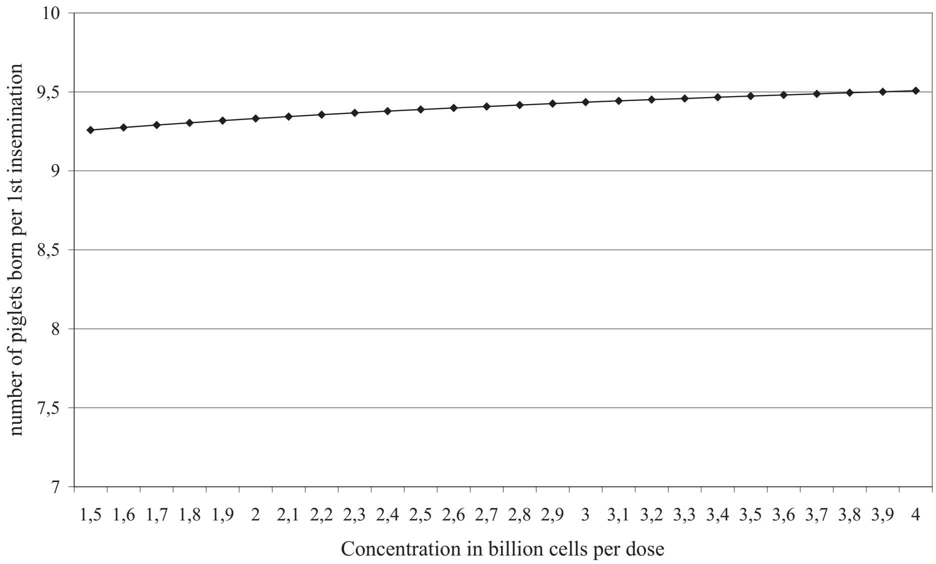
Graph 4. Relation between percentage abnormal cells and CASA motility score (R^2=0.188).

Effects of sperm number on fertility results

Over the years the total number of sperm cells in an insemination dose decreased in The Netherlands from 4.0 billion in 100 ml disposable bottles (1985) down to 1.5 billion in 80 ml disposable tubes (2008). This decrease has only been possible using strict production and processing conditions. In order to guarantee the quality of the insemination doses and the fertility results from the inseminate, the cooperative pig AI companies have a quality program based on HACCP (Hazard Analysis Critical Control Points). The quality and hygiene of semen production and processing are controlled in this way and when problems might occur, there is an action plan how to deal with these problems. Without these conditions it is not possible to dilute ejaculates that far.

Through the years experiments were performed using low dose inseminations. These data showed that it was possible to decrease the number of sperm cells in an insemination without noticing a significant effect on fertility results. Our analysis however of combined AI and breeding data showed a highly significant ($p<0.0001$) of number of sperm cells inseminated on total number of piglets born per first insemination [11]. Graph 5 illustrates the effect found on total piglets born per first insemination.

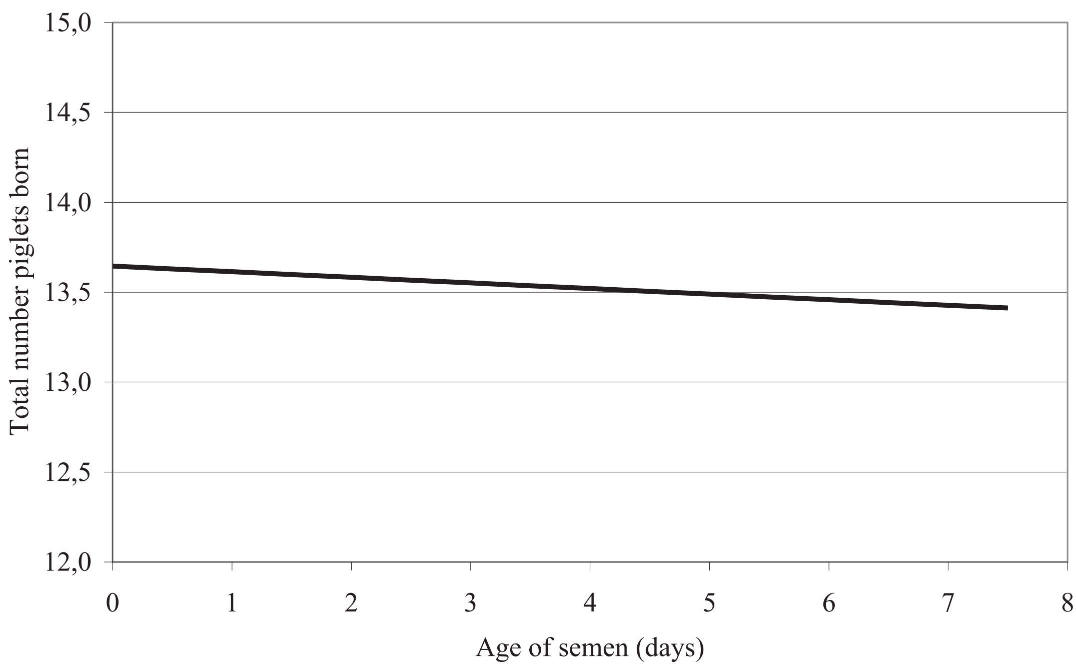
Although highly significant the effect is rather small (-0.07 piglet when decreasing the total number of sperm from 2.2 to 1.7 billion per dose). Only 6.7% of the variation in litter size is explained by the insemination dose used. Number of sperm cells in an insemination dose was only accounting for 1.2% of the explained variation. Factors as line of the boar, year and month of semen production and AI station are affecting the fertility results more (see paragraph on sources of variation). The economic effect on piglets produced in The Netherlands is about 2.5 million. In spite of the slightly negative result of decreasing the number of sperm cells, the consequence of sperm number reduction is that less boars can be used to produce the necessary insemination doses. Therefore the average genetic level of the boars present at AI stations will increase. The resulting economic effect was not calculated yet, but is assumed to overcome the negative economic effect of reduced sperm number.



Graph 5. Relation between total number of sperm per insemination dose on number of piglets born per litter per first insemination.

Effects of semen age on fertility results

Since the 1970-ties the pig AI companies in The Netherlands use BTS as the basis for semen dilution. Until 1994 the shelf life for semen was set on 48 hours. In the Netherlands – where the transport infrastructure is highly developed and distances from AI stations to sow farms are small– this was not a problem. However using the semen for a longer time will reduce transport costs and therefore is beneficial. In 1994 a modified BTS extender (Solusem®) was developed and introduced in the Dutch pig AI centres. Since then the shelf life was considered to be 72 hours. The effect of semen age on fertility traits was analysed for Solusem® based on the datasets available [7]. Graph 6 illustrates the result of this analysis, showing a significant effect (p=0.04) but relative small effect for increasing age of semen of -0.03 TNB per first insemination for each 24 hours the semen age increases.



Graph 6. Effect of age of semen on total number piglets born per first insemination.

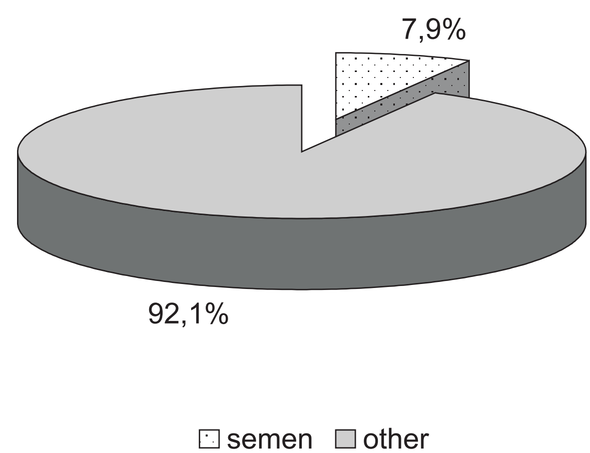
Before Dutch cooperative AI centres will change to another semen extender, scientific proof of increased shelf life without losses in fertility traits must be available. It is clear that, given the small effect, other extenders will have a hard time to prove to be better. Since the differences we are looking for are presumably small, trial size to compare extenders, must be large. Extender comparison will be discussed under future research and development.

Sources of variation in fertility traits and insemination doses

Litter size and farrowing rate are important fertility traits for the farm economy. When the technical results on farms are insufficient, often disease outbreak, feed or semen quality is blamed. Statistical analyses of PigBase data repeatedly show that variation in litter size is only for 4-7% caused by the semen component. A 10% of the variation is explained by the genetics of the sow, another 10% by the herd and 17% are fixed effects such as parity of the sow. This means that the role of semen in the fertility results is limited as long as the quality is good.

The Dutch cooperative pig AI centres stated that the semen should never be the limiting factor in the fertility results on the farm. Therefore more accurate analyses of semen data were performed. In the paragraphs above, several aspects of semen quality were discussed. The analyses performed in the past, led to statistical models which could predict fertility effects of motility, morphology and semen age. Besides predictive fertilising ability of semen, another aspect is important too for the management of pig AI centres. As showed in Graph 1, a significant effect of season (corrected for temperature) was found. Each analysis on semen quality data showed sources of variation for the trait being analysed [1,3,7,17]. Therefore these results create opportunities to improve semen quality by managerial measures at the pig AI stations.

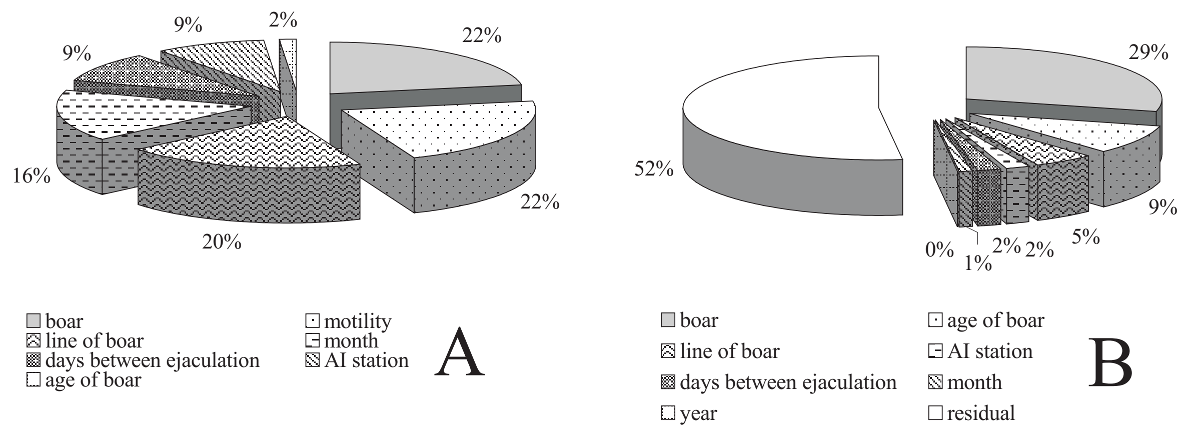
Graph 7 shows sources of variation in litter size based on the combined data of CASA motility results (2006 and 2007) and the related litter records from PigBase. Between different analyses, the absolute % of variation caused by semen may differ, but contribution of semen in the litter size variation varies between four and eight percent general.



Graph 7. Sources of variation in litter size (CASA motility data combined with related litter records). Other are other than semen such as sow, genetic line, herd and parity.

Which factors contribute to the semen based litter size variation, is shown in Graph 8, graph A and B are based on the statistical analyses of morphology and motility data respectively. Although the percentages may differ between the analyses, it is clear that the year and month of ejaculate production, the collection frequency and the AI station where the boar is located influence the semen quality.

AI centre management could for example decide to invest in temperature control at barn level to overcome the autumn dip in morphology. At the same time one could investigate differences between AI stations in for example housing and feeding of boars or in the way semen is collected and processed and how this relates to differences in morphological abnormalities in semen. At the same time boar related factors as: boar, line of the boar and age of the boar affect the morphology already for 43%. The management of an AI station could decide to cull boars over a certain age, or cull boars - repeatedly scoring high percentages of abnormal cells in their ejaculates - earlier than the current policy prescribes. This will in general lead to improvement of the AI stations performance.



Graph 8. Contribution of several factors to the semen based variation in litter size. A: analysis of motility data; B: analysis of Morphology data.

Not discussed in the earlier paragraphs are the sow factors related to fertility, such as oestric cycle, maturation of egg cells, ovulation, semen transport, semen selection at the utero tubal junction (UTJ) and the fertilising process. This work has been performed in the past and is still ongoing in close collaboration with Wageningen University and the Faculty of Veterinary Medicine of the University of Utrecht.

FUTURE RESEARCH AND DEVELOPMENT

For the future the Dutch cooperative pig AI centres foresee more research on semen quality characteristics and the relation with fertility.

Semen quality

Part of a PhD study is the validation of several flow cytometer tests, where fluorescent probes attach to specific structures in the sperm cell or at the sperm cell surface. Flow cytometers are capable of counting large number of cells in a relative short time span. Although a lot of these tests are claiming to indicate fertilising capacity of semen, it is never tested in a (large) boar population and analysed for their relation with fertility results [10]. The objective is to select the most predictive tests and develop them in a way they can be performed fast and easy in the AI laboratory.

As discussed in the part of the morphology analysis, abnormal semen cells have a negative impact on fertility results. Therefore fast detection of ejaculates with too many abnormal cells, is a requisite for pig AI centres. The development of an Automated Semen Morphology Assessment (ASMA) device or a software package using data from existing CASA systems is an important goal for the coming years.

Feeding for fertility

Two boar barns have been appointed at the Dutch cooperative pig AI centre to perform continuous feed trials in order to find the most effective feed composition to increase semen production, to improve semen quality and longevity. A lot of publications indicate these effects of special additions to feed or special feed composition. Trials performed by the AI centres in the past did not confirm these research results. Nevertheless feed quality is important since suboptimal body condition may impair the fertility results, hence the search for optimal feeding and fertility improving components will go on.

Semen extenders

A semen extender is the backbone of the insemination dose. The extender provides the semen with nutrients and keeps the pH on the adequate level. The composition of the extender is important for the longevity of semen. Depending on the country, longer shelf life in order to enable long distance transports, is important. In 2004, a project started for the development of a long term extender. The criteria for this long term extender are strict. Even if the preservation period is longer – aim is to preserve semen for at least five days - the fertility results may not be affected by it negatively. The development phase has now ended and the Dutch cooperative pig AI centres therefore are starting a large field trial. The set up enables the comparison of several extenders. Results are expected in 2010 and later.

CLOSING REMARKS

Since AI in pigs has become so important and sow farmers are depending on AI for the largest part, it is the responsibility of pig AI companies to ensure that the insemination doses produced are never a limiting factor for fertility results and that semen will not play a role in disease transfer. At the same time breeding companies must provide the top genetic boars to AI companies in their own interest. Current and future research must unravel the factors which are involved in fertilising ability of semen, the fertilisation process of the egg cell and early embryonic development and implantation, so variation in litter size will become smaller and technical results will improve even more. Next to specific focus on semen, sow related fertility aspects and prevention of disease transfer via AI are important as well, but not discussed in this paper. However, these areas are covered in the research of the Dutch cooperative pig AI centres.

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