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Artificial Insemination in Swine

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1. Introduction

The world population is 6.4 billion people approximately and is constantly growing. In this context, there is the expectation that it will reach 8.1 billion in 2030 and nine billion in 2050. In the next 25 years, this population growth will demand some 50% increase in food production. So, the world will be required some 53% increase in meat production, therefore elevating from 367 to 562 million tons. This will be necessary due the growth of the population and the increase of the per capita consumption, which is foreseen to reach 19.1Kg swine meat for inhabitant in 2030. So, the production of swine meat should present a growth around 20%, therefore reaching 155 million tons (Roppa, 2006). This growth is really happening, as considering that the world production of swine meat in 2010 reached 101 million tons, with projection of 133 million for 2019 (ABIPECS, 2011).

The increase of the productivity in the world swine confinement is happening along the last decades. According to data from ABIPECS (2011), China leads the world ranking by annually producing about 50 thousand tons of meat, as followed by the European Union, United States and Brazil (22,250, 10,052 and 3,170, respectively). This high production basically occurred by development and adoption of new technologies in practically all areas, such as genetics, nutrition, management, sanity and reproduction. Undoubtedly, in the reproduction area, the artificial insemination (AI) represents an enormous progress in production of swine. Since the beginning of the 70-ies, this technique provoked a great impact on increment of the swine production, especially in Europe and more recently in USA (Gerrits et al., 2005).

Initially, AI appeared in order to provide the genetic improvement of the animals and to solve sanitary problems. However, a significant improvement in both productive and economical aspects were later observed, as making possible an acceleration in diffusion of the desirable characteristics of the reproducers with high genetic value. This occurred due to AI great potential in making possible the use of biotechnologies such as those related to technology of the semen, preservation of embryos, and others.

The intracervical insemination (ICAI) is most used in the technified farms. Under the practical viewpoint, it is a simple and easily accomplished procedure. In this technique, the semen is deposited in cervix and the spermatozoids are transported until the ampulla of the uterine tube, the place where fecundation occurs (Rath, 2002).

More recently, new AI techniques in which the deposition of the semen is accomplished in uterus or in the uterine tube have been developed. So, there are intrauterine artificial insemination (IUAI), deep intrauterine artificial insemination (IUPAI) and the intratubal artificial insemination (IOAI) through laparoscopy. These new techniques are used in order to reduce the number of spermatozoids and the volume of the insemination dose. Many studies have been developed toward the improvement of those techniques, so that the spermatic concentration and the semen volume are maximally reduced, without negatively interfering in the reproductive efficiency. So, those techniques would make possible an increment in the genetic gain for reducing the cost of the dose and maximizing the use of the genetically superior males.

However, in spite of the relative simplicity of the AI in swine, there are several factors that direct or indirectly affect negatively the reproductive efficiency of inseminated sows. It is important to emphasize that many of those factors also interfere into reproductive efficiency of the sows submitted to natural mating.

In this chapter, the objective is to discuss the advantages, limitations and procedures of the AI. Besides, some important factors that direct or indirectly affect the reproductive efficiency of the swine herds.

2. The artificial insemination

According to the first reports, the use of AI in swine occurred in Russia and Japan (Ivanow, 1907; Nishikawa, 1964). Later, the AI diffusion was gradually happening in several countries. It is probable that the natural prolificacy of the swine species has delayed the development of the reproduction biotechnologies. However, the needs for genetic exchange and the sanitary pressures constituted a strong impulse for AI development.

In many countries, the AI growth is linked to expansion of the swine production at industrial scale. Considering the AI advantages, compared with the natural mating, the implantation of this one substantially facilitates the reproductive management of herds with high number of sows.

Most countries of the European Union adopt the AI at least in 60% of their females. In the last two decades, more than 90% swine females in the European west were artificially inseminated (Gerrits *et al.*, 2005). In Holland, for instance, more than 98% sows are artificially inseminated (Feitsma, 2009).

The AI use contributes for a larger sanitary control and hygienic cares in the matings. It also makes possible a better control of the semen quality due to rejection of inappropriate ejaculates. Besides those advantages, AI facilitates the management by the reduction of both time and work for mating. Another important aspect is the reproductive performance can be equal or superior to that obtained with the use of the natural mating.

This reproduction method presents great advantages compared to natural mating. In this context, the following advantages are distinguished: the genetic gains with the use of the genetically superior males, the reduction of the covering costs by female; and the decrease in the number of males in the farm. This last condition optimizes the use of the facilities.

Today, it is still possible to observe less technified farms that use the natural mating, which requires higher amount of males in a herd. This occurs because the male/female relationship for this condition to be a male for each 20 or 25 females, approximately. So, the producer will have higher expenses with facilities, feeding and medicines.

Despite all those advantages, however, the AI has some limitations in swine. In closed AI programs, in which the collection and processing of the semen are accomplished at the own farm, the investments in constructions and equipments are necessary for the installation of one semen production unit. In open programs, in which the doses are acquired from the centers external to farms, the main limitations are related to communication and to dose transports (Hansen, 2004).

Other limitations are also common in both programs, such as the need for maintaining the doses at temperature from 15°C to 18°C and the short storage period of the cooled doses (usually up to 72 hours). The reduced survival of the spermatozoids in the female genital organs is also a limiting factor. Besides those aspects, there are other factors such as the great variability in duration of the oestrum (from 12 to more than 96 hours) and at the moment of the ovulation among the swine females. However, the range of advantages obtained with AI undoubtedly overcomes the disadvantages of the same one.

2.1 Intracervical artificial insemination

The artificial intracervical insemination (ICAI) technique is the most used in technified farms. On the practical viewpoint, it is a simple and easily executed technology. The application of this technique optimizes the use of the males, which can supply up to 2,000 doses/year when under good management conditions (Bennemann et al., 2003).

In spite of this simplicity, a careful training of the employees and their understanding with reference to this technology are fundamentally important. Another relevant aspect is the way to implant the insemination technique in a farm. Since the year 1995, our work group already implanted ICAI in 69 farms at the states of Minas Gerais and Espírito Santo - Brazil. For this procedure, a transition period in the change of the natural mating to ICAI was defined. Our suggestion is the implantation to be partial, along approximately six months (transition phase). So, natural mating and inseminations occur weekly in the farm during this phase.

This condition makes possible to compare monthly the estrus replication between both methods. As soon the parturitions begin, the size of the litter will also be monitored. So, after approximately six months under evaluation, the decision for total implantation of the insemination is made. For this decision, the reproductive efficiency of the inseminated sows must be the same or superior to that of the sows submitted to natural mating.

The spermatic concentration required for ICAI are three billion spermatozoids. This concentration is important, as taking into account that in ICAI the semen is deposited in the cervix and its great part stays retained in the protuberances and cervical crypts. Then, these structures work as the first physical barriers to spermatic transport.

The swine specie is the only ones in which the volume of the insemination dose is as important as the spermatic concentration. In the other domestic species, the average or thin pallets (0,5 and 0,25mL, respectively) are generally used as containers for semen conditioning. In sow, the volume of the insemination dose used for ICAI is 80 to 100 mL. So, the semen recipients must have capacity to condition this volume. Very reduced volumes for ICAI can increase the rate of the estrus replication and/or to reduce the average number of pigs born by litter.

When the spermatozoids are deposited in the cervix, they are transported until the ampulla of the uterine tuba, where fecundation happens (Rath, 2002). For this condition, the spermatozoids find other physical barrier that is the uterutubal junction, which also works as spermatic reservoir (Langendijk et al., 2005).

Besides the barriers to be broken for the spermatozoid to reach the place of fecundation, other inconvenience of the insemination is the occurrence of seminal reflux. The inseminator' ability is fundamental to minimize the amount of reflux. However, only this ability has no effect, in case the inseminator is impatient. Besides, the time required for accomplishment of the insemination is an important factor. This makes sense, as considering that in the natural mating the penis introduced into female provokes the oxytocin liberation and, consequently, contributes to spermatic transport (Hafez, 2000). According to Langendijk et al. (2005), the IA pipette should remain in the animal'cervix during enough time for liberation of the oxytocin.

In this context, the recommendation by our work group is the insemination to be accomplished slowly, as maintaining the pipette fixed in cervix for approximately 10 minutes. Nowadays there are many available supports in the market, which are placed on the back of the sow for elevation and fixation of the pipette segment that is external to vagina, whereas the same one is fixed in the cervix. This way, the inseminator can inseminate other animals without the need for awaiting 10 minutes in each animal and later to begin IA in another animal.

In the ICAI technique, the semen is deposited in the first centimeters of the cervix. Due to anatomical characteristics of this structure, it acts as a natural barrier that hinders the arrival of the semen into uterus, therefore facilitating the occurrence of reflux through vagina.

The occurrence of reflux in the swine species is very common and it was observed in 100% animals inseminated by Steverink et al. (1998). According to those authors, the reflux presents differences in volume and in the spermatic concentration, according to each inseminated animal. However, some authors consider the reflux to be a physiologic event in the swine species. According to them, this reflux could only influence the fertility rate when the concentration of the insemination dose is equal or inferior to one billion spermatozoids, in 80 mL volume (Steverink et al. ,1998).

This spermatic concentration effect on fertility can be evidenced in the work by Watson & Behan (2002), When inseminating the females, those authors used three different spermatic concentrations (three, two and one billion spermatozoids) by ICAI and they concluded that the females inseminated with one billion spermatozoids presented low number of newborn piglets.

In a work carried out by our team, 120 females were inseminated by ICAI (Araújo et al., 2009), as being the semen reflux found in 100% females. The animals were observed up to 120 minutes after insemination. Some 100mL doses containing $3x10^9$ spermatozoids were used. The average volume of the reflux was 85.8mL, with a loss of 782.4 million spermatozoids by each IA. In this work, a relevant aspect is that insemination was carefully accomplished during a period of 10 minutes, by people highly expert in insemination. Surprisingly, animals with more than 105% reflux were observed, despite the cares previously mentioned, as indicating that secretions from the genital organs also constitute the volume reflowed. The reflux volume varied from 50 to 105%.

The ICAI allows for using the fresh, refrigerated or frozen semen. Concerning to fresh semen, it must be used immediately after its processing, without previous cooling. The cooling at temperature from 15^o to 18^oC is more used in both farm routines and insemination centers. It allows the maintenance of the spermatic viability for a period up to 72 hours.

Concerning to frozen semen, it was firstly used on the beginning of the 70-ies, as firstly with the insemination into uterine tuba and later with ICAI. There were progresses in using the frozen semen, due to researches accomplished with different cryoprotectors, conditioning packages, diluents, and freezing and defrosting curves. However, the use of the frozen semen in ICAI is still associated with the reduction from 10% to 20% in the parturition rate and from one to two piglets by litter, when compared to the use of refrigerated semen (Bernardi et al., 2005).

2.2 Intrauterine artificial insemination

In order to reduce the number of the spermatozoids/female/year, new techniques for artificial insemination were recently presented. Among them, the intrauterine artificial insemination (IUAI) through the use of the post-cervical probe is distinguished. This technique consists of deposition of the semen doses directly into body of the sow' uterus, from which the length is five to ten centimeters. The IUAI technique optimizes the semen production, as using low spermatic concentration by dose. This condition increases two to three times the number of doses by ejaculate.

The cost in maintenance of the semen donors includes the costs of the male acquisition cost, its depreciation, medicines, feeding and facilities. These expenses can represent 30 to 50% the total cost of the semen dose. In this sense, as higher is the number of the doses produced by each housed male, the higher will be the efficiency and lower the cost (Bennemann et al., 2003; Weber et al., 2003; Hansen, 2004). Whereas the traditional insemination requires one boar for each 100 to 150 female, one boar can attend up to 450 females in the intrauterine insemination, approximately.

Taking into account the better use of the ejaculate in IUAI, it is also distinguished the possibility to increase the selection intensity in the females production, by using the genetically superior males. Evidently, this condition would not be applied at commercial farms, from which the purpose is the production of animals for slaughter. In addition, this is a very useful technique for the researches with swine frozen semen, as taking into account that the IUAI, the spermatic volume and concentration are more reduced than in ICAI.

At first, IUAI is a higher perilous technique, as taking into account the impossibility to fix the cervix by hand, such as in cow or even retracting towards the outside of the vagina, as performed on goats and sheep. So, many technicians consider its implantation to be difficult in commercial farms. However, a work carried out by our group (Araújo et al., 2009) demonstrated the opposite. In this work, the ICAI techniques were compared to IUAI. ICAI was performed using a Melrose (Minitub®) pipette. IUAI was performed using an intrauterine catheter "Verona" (Minitub®). Despite the difficult passage of the pipette in 4.6% females submitted to IUAI, 100% of those females were inseminated (Table 1).

Description	Insemination technique	
	Intracervical	Intrauterine
Number of inseminations	120	480
Difficulty to introduce (n)	0^a	22^b

Table 1. Degree of dificulty to introduce the pipete in females for intracervical insemination (n=120) and intrauterine (n=480). A difference (P<0.05) was found between the insemination techniques by the chi-square test. Adapted from Araújo et al. (2009)

The difficult passage of the pipette in some animals is due to IUAI to be more invasive. In the present study, however, the catheter was introduced into uterus and the insemination happened successfully in all of the animals. However, Diehl et al. (2006) observed to be impossible the introduction of the catheter into uterus of 4.5% females. This difficulty probably occurred due to the short insemination time used by the authors (average: 2.3 minutes/insemination), especially in primiparous females, where there was higher number of animals in which there was difficulty in introduction of the catheter. In those cases, while the catheter is introduced, either inseminator's patience and the constant stimulation of the female by massage on lumbar area allow the success of the technique.

It is important to emphasize the possibility for occurrence of bleeding during the introduction of the insemination pipette, a condition verified by Watson & Behan (2002). This is due to factors such as the technical ability of the person responsible for insemination as well as the pipette type. Another interfering factor is the speed in introduction of the pipette, since as higher is the insemination speed as higher will be the bleeding possibility (Diehl et al., 2006). Besides those factors, the females with higher parturition number present larger development of the genital organs than the primiparas or nulliparas. Thus, it is easier the introduction of the catheter into cervix, therefore reducing the incidence of lesions.

However, the occurrence of bleeding during insemination does not affect the reproductive efficiency. This condition was verified by our work group, as the ICAI was compared with IUAI (Table 2). The presence of blood was observed in 1.6 and 7.7% of the animals inseminated via ICAI and IUAI, respectively. Nevertheless, this bleeding did not influence the estrum replication rate neither the total newborns by litter. Those results corroborate the by Watson and Behan (2002), who did not observe any deficit in the reproductive efficiency of the sows that presented bleeding after IUAI.

Insemination technique	Presence of blood	Number of Inseminations	Number of sows	Return to estrus rate[1]	Litter size per parity
Intracervical	Without	118	58	4.2	11.7 ± 3.2
	With[2]	02	02	0.0	9.5 ± 9.1
Intrauterine	Without	443	211	4.5	11.6 ± 3.1
	With	37	29	5.4	10.8 ± 4.3

Table 2. Return to estrus rate and litter size per parity with inseminations in the presence and absence of blood. Adapted from Araújo et al. (2009). No differences (P>0.05) found between the insemination techniques by the Chi-square test for return to estrus rate. No differences (P>0.05) between the insemination techniques (Duncan Test) for litter size mean per parity. [1]Return to estrus rate percentage of total number of inseminated sows for each insemination technique. [2]Not evaluated statistically due to the reduced number of occurrences.

The ICAI is known as technique presenting considerable vulvar reflux of the semen after AI. However, our work group (Araújo et al., 2009) verified that such a fact also happens with IUAI. In this experiment, we verified the semen reflux to occur in practically all animals, independent of the technique used (100 and 98% for ICAI and IUAI, respectively (Table 3). On the other hand, some works do not mention the presence of reflux in IUAI, perhaps because they only observed the first instants after AI (Benneman et al., 2004; Mezalira et al., 2005), differently of our work, in that the animals were observed until 120 minutes post IUAI, once the reflux does not occur right after insemination.

Insemination technique	Number of inseminations	Semen backflow rate (n)
Intracervical	120	100% (120)
Intrauterine	480	98% (471)

Table 3. Semen backflow in the inseminations according to the different insemination techniques. No differences (P>0.05) found between insemination techniques by the Chi-square test. Adapted from Araújo et al. (2009).

The occurrence of the semen reflux can have negative effects on the reproductive efficiency, such as the losses of spermatozoids. This condition is based on the fact that here is a minimum number of spermatozoids by dose, for the maximum reproductive efficiency. However, despite the high occurrence of semen reflux found by our work group, no negative effects occur in the return rate to estrum and in litter size (table 4).

Insemination technique	Backflow	Number of inseminations	Return to estrus rate	Litter size per parity
Intracervical	With	120	5.0%	11.56 ± 3.4
Intrauterine	With	471	4.0%	11.48 ± 3.3

Table 4. Return to estrus rate and litter size per parity in inseminations with backflow according to the different insemination techniques. No differences (P>0.05) found between the insemination techniques by the Chi-square test for the return to estrus rate. No differences (P>0.05) found between the insemination techniques by the F test for litter size per parity (Adapted from Araújo et al., 2009).

It is evident the spermatozoids number and the insemination dose volume are decisive factors for the volume reflux to interfere in the reproductive efficiency. In the experiment carried out by our group (Araújo et al.,2009), IUAI was compared with ICAI, as confronting two insemination volumes (100 vs. 50mL) and different concentrations of spermatozoids. Although the volume of the semen reflux has been similar among the treatments (P>0.05), the amount of spermatozoids of the reflux in females receiving IUAI was smaller (Table 5).

Insemination technique	Number of spermatozoids	Backflow volume in mL (%[1])	Total of backflow sptz in millions (%[1])	Number of backflows collected
Intracervical	$3x10^9$/100 mL	85.8 (85.8%)	782.4 (26.0%) [a]	23
Intrauterine	$1x10^9$/100 mL	83.2 (83.2%)	164.0 (16.4%)[b]	25
Intrauterine	$1x10^9$ /50 mL	41.5 (83.0%)	111.4 (11.1%)[b]	25
Intrauterine	$5x10^8$/100 mL	87.8 (87.8%)	80.5 (16.1%)[b]	28
Intrauterine	$5x10^8$/50 mL	45.3 (90.6%)	58.0 (11.6%)[b]	30

Table 5. Total number of spermatozoa during backflow in millions and number of backflows collected using the different insemination techniques. [1] Correspond to percentage in the reflux, as considering the volume or the total number of spermatozoids of the insemination dose. No differences (P>0.05) occurred between the insemination techniques by the Kruskal - Wallis test, concerning to the collected volume. There was difference (P<0.05) between the ICAI technique in relation to IUAI by the Kruskal - Wallis test, concerning to spermatic concentration. Adapted from Araújo et al. (2009).

This occurred because the semen is deposited at the third initial/medium of the uterus, as probably facilitating the fast progression of the spermatozoids toward the spermatic reservations, therefore allowing a high retention of cells in the genital organs (Dallanora et al., 2004).

Taking into account the advantages of IUAI, many researchers have been accomplished in the last years, in order to define the spermatic concentration and the ideal insemination volume for maximization of the results by using this technique. So, Dollanora et al. (2004) compared the use of ICAI (three billion spermatozoids at 90mL doses) with IUAI (1.5 billion spermatozoids at 60mL doses). Those authors obtained no differences between both treatments for the adjusted childbirth rate and total number piglets born.

When comparing ICAI (three billion spermatozoids in 100ML doses) with IUAI (1 billion spermatozoids in 50mL), Sumransap et al. (2007) verified there were no differences among the total number of spermatic cells in different segments of the genital organs from the most caudal area of the uterus until the ampulla of the uterine tuba**.** Thus, even with the reduced number of spermatozoids in the dose, IUAI provides the same number of spermatic cells in the spermatic reservoirs.

However, highly reduced concentrations of spermatozoids in the insemination dose (250 million) can reduce the size of the litter, by reducing the spermatic reserves (Mezalira et al., 2005).

The volume of the insemination dose is also a decisive factor in the reproductive efficiency of the herd. In this context, some works report that IUAI accomplished with highly reduced volume endangers the reproductive efficiency of the herd. This is evident in the work by Bennemann et al. (2005) who used IUAI with 500 million spermatozoids by dose, in volume of 20 mL (154 sows), as comparing with ICAI with three billion spermatozoids in 90 mL (144 sows). The farrowing rate did not differ between treatments. When using IUAI, however, a significant reduction occurred in the total number of born pigs.

The experience of our work group (Araújo et al., 2009) shows that the use of $5x10^8$ spermatozoids in 50mL can adequately substitute the traditional technique (ICAI) without endangering the reproductive efficiency of the inseminated animals (Table 6). It is probable that the use of the oxytocin in semen has contributed to those positive results. In works accomplished by our research group, the addition of 2.5 UI oxytocin at the insemination dose of 100 mL does not interfere in the physical parameters of the semen and morphological ones of the spermatozoids (Podda et al., 1999), as well as it does not endanger the replication rate of estrous. Additionally, the oxytocin in this preconized dose increases the size of the litter (Costa et al., 1999). With the physiologic role to promoting the contraction of the flat musculature of the uterus (Bevan, 1979), the oxytocin can facilitate the ascension of higher number of spermatozoids until the fecundation site, taking into account that only a small proportion of the spermatozoids deposited during natural mating or insemination reach the distal portion of the uterine tuba.

In spite of those positive results found in this experiment, in which the insemination was accomplished by the same employees who performed the insemination routine, we still did not implant the IUAI with $5x10^8$ spermatozoids/50mL in the routine of commercial farms. However, Since the year 2007, our work group implanted the IUAI with $1x10^9$ espermatozoids/100mL, by using two inseminations (at zero and 24 hours after the beginning of the estrus) in 100% primiparous and pluriparous females at four farms (total of 2.500 females). No nuliparous females exist in those farms. The primiparas are proceeding from other farm of the same company. It is important to emphasize that the inseminations are accomplished by employees of the farms. This condition reinforces our position that the IUAI technique can be accomplished at commercial farms by the own employees responsible for the gestation sector.

Insemination technique	Spermatozoid number	Farrowing rate ([1]n)	Estrus repetition rate	Number of newborns by farrowing
Intracervical	$3x10^9$/100 mL	90.0 (54)	10.0	11.5 ± 3,4
Intrauterine	$1x10^9$/100 mL	93.3 (56)	6.7	11.7 ± 3,4
Intrauterine	$1x10^9$ /50 mL	86.7 (52)	13.3	11.4 ± 3,2
Intrauterine	$5x10^8$ /100 mL	93.3 (56)	6.7	11.8 ± 3,0
Intrauterine	$5x10^8$ /50 mL	90.0 (54)	10.0	11.4 ± 3,6

Table 6. Farrowing, estrus repetition rates and total piglets born per farrowing in each insemination technique (60 females per treatment). [1]n: Number of animals which gave birth according to each insemination technique. No differences (P>0.05) occurred between the insemination techniques for farrowing and estrus repetition rates by the chi-square test. No differences occurred (P>0.05) between the insemination techniques for number of newborns by farrowing using Kruskal – Wallis test. Adapted from Araújo et al. (2009).

2.3 Deep intrauterine artificial and intratubal Inseminations

In the last years, many researches concerning to the deep intrauterine insemination (IUPAI) have been accomplished. In this technique, a low insemination volume (5mL) is used as well as reduced concentration of spermatozoids (200 million), without the need for surgical intervention (Vazquez et al., 2000). The objective of the researches accomplished until the moment is to turn this technique applicable (not endanger the reproductive efficiency of the herd, so that it can be commercially implanted at large scale. In addition, the use of reduced volume and low concentration of the sperm in IUPAI will favor the use of frozen semen and/or sexed.

The reduction of the semen volume used in IUPAI rather guarantees the optimization of the boar, as providing economical advantages to the farms. The possibility for using this technique is in line with the needs imposed by modern swine raise, which looks for reducing the insemination dose under use. This would provide a reduction in the male breeding stock and even in the frequency of using these ones.

It is considered that a great number of the spermatozoids are lost in ICAI process (Martínez et al. 2001). This occurs due to the semen reflux as well as to spermatozoid phagocytosis by the polymorphonuclear leucocytes. It is believed that approximately 1/3 of the spermatozoids by backflow in 2 hours after AI, due to those physiologic processes (Viring & Einarsson, 1981). After overcoming those obstacles, approximately 1 X 10^3 spermatozoids can be rescued at the caudal portion of the isthmus, a place where the spermatic cell stays until ovulation to occur (Mburu et al., 1996).

In this context, the IUPAI objective is the reduction of the spermatic flow inside the uterus, as reducing the seminal reflux and the phagocytosis rate on those cells (Vazquez et al., 2008). In addition, some physical barriers are transposed as the cervical folds and endometrial crypts. Thus, the insemination dose under use could be significantly reduced. So, Martínez et al. (2002) verified that IUPAI with 5 x 10^7 spermatozoids by dose (5mL) presents no differences in the gestation and parturition rates neither in the size of the litter, when compared with ICAI by using 3 x 10^9 spermatozoids (100mL). However, it is

important to emphasize that the control group (n=147) presented low rate for either parturition (83%) and for those born by litter (9.97), although those researchers used a high number of animals by treatment. Other aspect to be considered is that the estrum of the females submitted to IUPAI was induced, whereas the estrum of the control group was not.
The IUPAI technique consists of using a special pipette, which is fixed into cervix as in ICAI. Successively, a flexible catheter with 1.8m length is inserted through pipette along the cervical canal until reaching the final portion of the uterine horn. This technique provides the deposition of the semen in one of the uterine horns near the fertilization place.
The main IUPAI obstacle is the anatomical complexity of the sow´s genital organs. The cervical channel is characterized by presence of the cervical folds and uterine horns due to long length and naturally rolled. These characteristics delayed the development of a catheter for nonsurgical insertion in the uterine horns. So, Vazquez et al. (2005) report that, in 1999, they developed a nonsurgical catheterization technique for access to the uterus, by using a modified endoscope provided of flexible 1.35m optic fiber. Those researchers report the success in accomplishing this procedure.
Thus, the first accomplished IUPAI were based on the use of an endoscope at extremity of the insemination pipette, therefore allowing the visualization of either cervical channel and uterine horns. This technique associated with induction of the hormonal ovulation in sows has been making possible the deep deposition of the spermatozoid into uterus. It was demonstrated that the passage of the pipette associated with endoscope, along the cervical channel and uterine horn, is a simple process to be accomplished, as lasting 4.1 minutes on average (Martínez et al. 2001). Those authors show that the endoscopic IUPAI generates interesting results, such as parturition rates of 86.6%, 88.9% and 92.3%, by using 100, 20 or 5 x 10^7 spermatozoids in 5 mL of diluter, respectively. The average size of the litter was 9.41. Those data do not differ from ICAI (n=48) with 3 x 10^9 in 100mL. However, it is important to emphasize that the authors used a small number of animals (15, 18 and 13 females, respectively) for IUPAI. Besides, those animals were submitted to hormonal synchronization procedures, what could make unfeasible the use of this technique routinely in the commercial farms due to high cost.
The use of the endoscope represented a great progress in the procedure of the artificial insemination in swine. Due to deposition of the semen at proximities of the fecundation place, the IUPAI technique makes possible the use of the processed and weakened spermatozoids proceeding from cooling, freezing or sexing (Vazquez et al., 2005). However, the limitation of this technique is the cost of the equipment and its fragility. Thus, its use would not be applicable at field (Vazquez et al., 2005). From this verification, a number of researches were developed in order to eliminate the use of the endoscope in this procedure. This situation required the development of new IUPAI pipettes.
The proof of the IAIUP efficiency at field, without using the laparoscopy, was later confirmed by Martínez *et al*., 2005b. This author demonstrated that the fertilization rate of the sows inseminated with 150 x 10^6 spermatozoids diluted into 5ml BTS did not differ from that when the animals were inseminated with 3 x 10^9 spermatozoids diluted into 100ml of the same diluent through IAIC. However, some 10.9 reduction in size of the litter were observed in the conventional IA for 9.8 piglets in IAIUP. Based on these results, the authors verified the IAIUP application in commercial farms to depend on the proof that this technique will not endanger the number of the piglets born by parturition.
With the progress of the researches, the number of spermatozoids used in IUPAI were twenty times reduced for refrigerated semen and up to six times for frozen semen, in

comparison with ICAI. In relation to volume, the decrease resulting from the use of IUPAI was 8 to 20 times lower compared to ICAI (Vazquez et al., 2008).

However, the recurring concern of the researchers refers to unilateral fertilization. Although small semen doses are only deposited in an uterine horn, the bilateral fertilization was proven in approximately 100% of the cases, according to either Martínez et al. (2002) who used the IUPAI with endoscopic catheter and Tummaruck et al. (2007) by using IUPAI with catheter without endoscope. Those authors did not find significant difference in the number of embryos found on each side. For this evaluation, they slaughtered the sows at approximately 60 hours after IUPAI. Then, they evaluated the washed of the uterine tuba and extremity of the horns.

However, a detailed study by Martínez et al. (2006) showed that, in sows ovulating spontaneously (without induced ovulation), the bilateral fertilization that is, in both uterine horns, is not 100% effective. For this confirmation, those authors used IUPAI with doses of $0.15x10^9$ spermatozoids/ 20 mL. So, those researchers verified that 21% sows submitted to IUPAI presented unilateral fertilization. In addition, 15.8% sows presented partial bilateral fertilization. Those researchers also found differences regarding to the rate of normal embryos in the horn with less embryos, when comparing IUPAI with ICAI ($2.95x10^9$ spermatozoids/95mL). Corroborating with those authors, Buranaamnuay et al. (2011) demonstrated that, from a total of five inseminated animals, three presented unilateral fertilization. The animals were submitted to IUPAI procedure without laparoscopy, as using 1×10^9 defrosted spermatozoids.

Those contradictory discoveries suggest the mechanism in which the spermatozoid reaches the counterlateral horn still stays obscure and needs to be more studied, in spite of the evidences for trans-uterine and trans-peritoneal migration (Martínez et al. 2005a; Tummaruck et al. 2007).

The IUPAI will represent a great economical advantage to the farm, since it will reduce the costs with acquisition of males, ration, medicines, vaccines, management. Besides, it would guarantee a larger uniformization of both herd and litter. However, the high cost of the pipette for this procedure and the difficulties in execution of the technique still represent impediments for its implantation in commercial farms. To these factors are added the results still inferior in reproductive efficiency, when compared with ICAI and IUAI.

Thus, it is clear that IUPAI represents a technique with a promising future to be commercially used at the farms, since the costs are decreased and the reproductive efficiency is not endangered.

Another developed technique is the artificial intratubal insemination through laparoscopy (ITAI). This new technology attends the premise to use doses at much reduced spermatic concentrations and at small volumes. Above all in specific situations, when the use of frozen, sexed semen or the genetically modified semen is proposed, the low number of viable spermatozoids can be compensated by deposition of the close semen or the semen inside the uterine tuba, then obtaining satisfactory fertilization rates.

The ITAI allows the inseminator, with the aid of a laparoscope, to determine the time and the ideal place for deposition of the semen, as reducing the exhibition of the spermatic cells to adversities and positioning them close to the uterus-tubaric junction.

For execution of this technique (Vazquez et al., 2008), initially the animal is placed in Trendelemburg position (that is, dorsal decubitus with the head side lightly sloping) at 20° angle with the horizontal. Successively, an incision close to 1.5cm near the navel is accomplished. The borders of the incision are tractioned and a optiview trocar associated with a laparoscope is inserted into incision, as this being removed later. So, the access to the abdominal cavity is possible with laparoscope. The abdominal cavity is inflated with CO_2 and two lateral openings are accomplished in the hemi-abdomen for the access of the Forceps tweezers. Those tweezers aid in the manipulation and fixation of the uterine horns and uterine tubas for the introduction of the insemination needle. After accomplishing the procedure, the tweezers are removed and a small suture is necessary. The procedure takes approximately 15 minutes.

Laparoscopy is considered a less invasive technique than laparatomy for introduction of the semen into uterus or in the uterine tuba (Vazquez et al., 2008), since laparatomy can cause either higher stress to the animal and adherences at the postoperative period, therefore prejudicing the future inseminations (Fantinati et al., 2005).

The insemination by ITAI makes possible the use of doses as low as five million spermatozoids in 0.5mL (refrigerated semen) in each uterine horn, without affecting the efficiency of the fertilization and production of piglets, when associated with laparoscopy (Fantinati et al., 2005). According this author, however, this technique should be obligatorily accomplished in both horns, since the low concentration, the small volume and the deposition in a precise place impede the spermatozoid to migrate and reach the collateral horn.

Due to high number of spermatozoids introduced into uterine tuba (3-6 x 10^5) during ITAI, special attention should be taken with regard to polyspermy. The polyspermy is affected by the spermatozoid: oocytes proportion and by the insemination moment, as this one is related to modifications in the environment of the uterine tuba. In this context, Vazquez et al. (2008) verified the polyspermy incidence to be very low, when spermatic concentrations of $3x10^5$ and $5x10^5$ are used by dose or when the sows are inseminated before ovulation. Otherwise, when those animals are inseminated with 1 x 10^6 spermatozoids/dose or during the preovulatory period, the possibilities for polyspermy are increased. Thus, low spermatozoid concentrations ($3x10^5$) were shown to be effective when used before ovulation in ITAI, as opening possibility for use of the sexed and frozen semen.

When working with the dose of 5 x 10^6 spermatozoids/0.5mL in each horn in the laparoscopic IAITU and 3 x 10^9 in ICAI, Fantinati et al. (2005) obtained high fertilization rates of oocytes and developmental competence of the embryos, which were collected and cultivated *in vitro*. However, it is worth to emphasize that the females had the estrum induced with eCG and hCG.

Although this technique was commercially applied in sheep (Anel et al., 2006), in swine it is still limited to experimental assays. The highest difficulties for its commercial accomplishment are the need for personal training, structure and specialized equipments.

2.4 Some factors affecting the reproductive performance of inseminated females

Several aspects are able to interfere into results of the AI programs. However, it is important to emphasize that many of those factors also interfere in the reproductive efficiency of sows submitted to natural mating. The objective of this topic is to distinguish some important aspects in this context.

In the last 25 years, the AI in swine was expressively desenvolved, as contributing to genetic improvement of the herd and increase in production. During this period, a reduction in the number of spermatozoids by each dose, that passed from $6x10^9$ to up $5x10^4$. At the same time, the useful life of the semen increased considerably, as turning more flexible and practicable the process (Waberski et al., 2008).

The use of reduced spermatic concentrations in the insemination doses comes to encounter the premises of the swine confinement of the XXI century. To attend those requirements, however, it is necessary a high-qualified semen, therefore guaranteeing a high reproductive efficiency. In addition, to assure this condition, it is necessary an effective quality control and the monitorship of several factors that can interfere into results.

In this context, several factors are very important such as: the action of the pathogenic microorganisms, nutritional conditions, the age of the first mating, the lactation period, the seasonal influence and the management in detecting the estrus detection.

The reproductive efficiency of the artificially inseminated sows is extremely affected when a contaminated semen is used. The microbial contamination of the semen can result into reduced reproductive efficiency due to low seminal quality, precocious embryonic death, and endometritis (Guerín & Pozzi, 2005).

The forms of the semen contamination can be classified as being from animal origin or not. The contamination from animal origin is due to infection of the boar, proceeding from the testicles or other segments of the genital organs. In addition, a number of contaminations may occur by breathing secretions or feces, which happen during the collection process and semen processing. Otherwise, the contamination not arising from the boars can occur, in most cases inadvertently during manipulation of the semen by the person responsible for the collect. Another responsible factor would be the excessive and mistaken exposure of the material collected at the air, skin and breathing secretions. Besides those aspects, the semen can be also contaminated by the quality of the water used during the processing, the ventilation system, sinks and drains (Maes et al., 2008).

A second factor affecting the reproductive performance of the animals is feeding. The supply of a ration that is in perfect balance of nutrients is essential. For this reason, it is fundamental the producer to receive technical orientation of the professionals specialized in the animal nutrition area.

The age of the animal at the first natural mating is also an important factor that should be taken into account. Usually, the sows present the first estrus at the age of five or six months. However, it is not indicated those animals to be inseminated before the seventh or eighth month. On this occasion, they will be weighing approximately 130 to 140kg on average, as depending on the female' genetics, and they will be presenting the third estrus. This fact is based on verification by Martín Rillo et al. (2001) who confirmed that the length of the female' genital organs during the first natural mating is directly proportional to dimension of the animal. Additionally, those authors found correlation between the length of the vagina and the length of the uterus. They still verified a larger size of the litter in animals presenting more developed genital organs at the action of insemination.

Another factor affecting the reproductive efficiency of the inseminated female is the nursing period. In the past, it was consensus that a shorter nursing period would increase the number of piglet births by year and, consequently, larger number of parturition by year and consequently higher piglets/sow/year. However, this theory was mistaken, because based on survey of 79,729 parturitions, our work group observed that as longer is the length of the lactational period as larger is the size of the following litter (Table 8). Thus, lactational periods from 22 to 25 days result in approximately one more pig in the following litter, compared with periods from 8 to 13 days. This condition was verified either in primiparous and pluriparous sows (Costa et al., 2004).

Lactation length (days)	Primiparous		Pluriparous	
	N	LS	N	LS
8 to 13	1,074	10.34 ± 0.9[a]	3,513	10.70 ± 0.5[a]
14 and 15	2,911	10.41 ± 0.5[a]	13,951	11.16 ± 0.2[b]
16 and 17	2,249	10.46 ± 0.6[a]	16,095	11.15 ± 0.2[b]
18 to 21	2,987	10.68 ± 0.5[b]	24,069	11.34 ± 0.1[c]
22 to 25	1,186	11.43 ± 0.8[c]	8,692	11.87 ± 0.3[d]
Total	10,867	10.86 ± 0.3	68,862	11.44 ± 0.1

Table 8. Average of litter size (LS) of primiparous and pluriparous sows submitted to different lactation lengths and respective parity number (N). Averages with different letters in the same column differ (P<0.05) by the Duncan test (Costa et al., 2004).

According to Koketsu *et al*. (1997), the uterine retrogressive development is not essential for establishment of the next gestation. In sow, however, the complete recovery of the endometrium happens between the second and third postpartum week (Hafez, 2000). This explains (Table 8) the influence of the nursing period on size of the litter only from 18-21 days under nursing in primiparous sows.

In pluriparous sows, however, a nursing from 14 to 15 days shows a significant increase in size of the litter, as compared with the precocious weaning (8-13 days), whereas primiparous females did not present this condition. Those results show the *post-partum* recovery to be less efficient in primiparous gilts, probably due to higher weakening of those animals. According to Koketsu et al. (1997a,b) the adverse effects of a short nursing period on the subsequent reproductive efficiency is less intense in females that maintain high ration consumption during nursing.

So, the uterine involution could be the main factor responsible for the low embryonic survival and, consequently, the reduced size of the litter in females submitted to the precocious weaning (Allrich et al., 1979; Foxcroft and Aherne, 2000; Machado et al., 2000). Associated the those factors, the presence of lochia can hinder the embryonic implantation and provoke the death of the embryos (Grunert and Birgel, 1982).

Our work group also evaluated the influence of the nursing period on the weaning-insemination interval (Table 9). Those discoveries demonstrate that a nursing period of 22 days at least provides a shorter weaning-insemination, interval, then decreasing the unproductive days of the sow.

Lactation length (days)	Primiparous		Pluriparous	
	N	WCI	N	WCI
8 to 13	1,074	5.63 ± 0.5[a]	3,513	4.94 ± 0.8[a]
14 and 15	2,911	5.43 ± 0.3[b]	13,951	4.60 ± 0.1[b]
16 and 17	2,249	5.07 ± 0.3[c]	16,095	4.44 ± 0.1[c]
18 to 21	2,987	4.80 ± 0.3[d]	24,069	4.38 ± 0.1[d]
22 to 25	1,186	4.67 ± 0.5[e]	8,692	4.31 ± 0.1[e]
Total	10,867	5.11 ± 0.2	68,862	4.54 ± 0.8

Tabela 9. Weaning-conception interval (WCI, average ± standard deviation) in 79,729 parturitions in pluriparous and primiparous sows with lactation periods of different lengths. (Amaral Filha et al., 2004). Averages with different letters in the same column differ (P<0.05) by the Duncan test.

Another aspect that should be observed in artificial insemination programs is the thermal condition of the environment to which the animal is submitted. The high temperatures reduce the efficiency of the heat loss, as making the animal to enter a hyperthermal state. This condition leads to embryonic mortality at the initial gestation stage. A survey accomplished with 100,934 parturitions in a tropical climate area shows the size of the litter to be significantly smaller in the hottest months of the year (Table 10). In the same way, in temperate area (Mediterranean conditions), the efficiency of the IA (parturition rate and litter size) is lower in summer-autumn season. Additionally, the administration of exogenous hormones (eCG and hCG) in the attempt to improve the ovulation rate proved to have no effects during this period (Bolarín et al., 2009).

However, when the environmental temperature becomes a restrictive factor for the embryonic viability, there are some alternatives to minimize the thermal effect. The ventilation, the floor cooling and the use of nebulization could partially reduce the adverse effect of the temperature on sow. This practice is applicable, mainly in tropical countries where the hangars for animals are open and exposed to adverse effects of the climate. Adult animals can have their critical temperature increased, that is, their resistance to heat is increased up to 2°C when they are submitted to ventilation from fans within facilities (Nääs, 2000).

Another aspect to be considered in AI is the efficiency in detecting the estrus. For the obtainment of indexes compatible with the goals established by the reproductive program, it is necessary to observe the IA ideal moment, as considering both estrus and ovulation. This condition is important, since a long IA - ovulation interval reduces the gestation rate, the embryonic survival and the litter size (Spencer et al., 2010).

The insemination protocol (AI moment) is defined as a function of the estrus beginning. Thus, more important than to find a sow under estrus is to detect the beginning of the same one. However, even with a good management in detection of the estrus, many times the beginning of this one is not characterized, taking into account that it might have happened during night. This fact can be the responsible for the highest incidence of the estrus detected at the beginning of the morning and not during afternoon. In a study conducted by our work group (Pinheiro, 2000), we verified that 16.66% of the estrus were initially detected at 15:30 hours. However, at 7:30 and 23:30 the estrus were detected in 44.44 and 38.88% of the animals. Therefore, 83.24% initial detections of the estrus occur in the morning, taking into account there is no routine at the farms for night detection.

Considering the importance of the initial detection of the estrus, it is worth to emphasize that some animals do not accept promptly the natural mating even when they are in estrus. However, when insisting with detection incentive, the animal presents the immobility reflex. So, it is very important a careful detection, mainly in females that already present modifications such as the vulva edema.

When the gilts are housed in stalls, the introduction of the teaser is recommended in those stalls, on the beginning of the morning and on the end of the afternoon, in order to detect the females in estrus. Those females presenting immobility to natural mating, a behavior known as reflex of tolerance to male (RTM), are considered to be in estrus. However, special attention should be given when RTM does not occur in females that already present modifications such as the vulva edema. In this case, we recommend to take the female to stall of the teaser it can be carefully evaluated.

Though, the management of the estrus detection in primiparous and pluriparous females are differentiated. After weaning of the litter, the females are housed in collective stalls or individual cages. Usually, the beginning of the estrus happens from the third and fourth day after weaning. However, RTM should be made already at the following day after weaning, at the beginning of the morning and final of the afternoon. This procedure is important, taking into account that some sows can advance the beginning of the estrus, in other words, at the first or second day after weaning.

When the females are housed in cages, the teaser is conducted in the corridor of the hangar, so he has contact with the sow. At this moment, an employee stimulates the sow by pressuring the back or even mounting on the same one. From the third day from weaning, the females presenting no characteristic behavior of estrus should be individually taken to the stall of the teaser. For standardization either in gilts and primiparous and pluriparous, the zero hour of the estrus is the moment at which the female presents RTM for the first time.

Another procedure used in practice for detection of the estrus is the reflex of tolerance to the man (RTH). This reflex is the result of the man-animal interaction, without the presence of a male. However, the results are various and inconsistent. A study carried out by our work team (Pinheiro, 2000) showed that 23% sows in estrus do not present RTH. For this study, the estrus was confirmed by RTM, besides the occurrence of ovulation that was confirmed by ultrasonography. The detection of the estrus was accomplished at 8hrs intervals (7:30, 15:30 and 23:30). The RTH reflex was accomplished before RTM, as considering that many sows in estrus can present positive RTH after they were previously sensitized by the contact with male.

We also verified that 44% females, which were in estrus, presented very short RTH (less than 16 hours). Those considerations were corroborated by DIAS et al. (1999), who found a very varied RTH period. Those researchers observed that 11% animals presented no RTH and 26.5% presented it for a period lower than 16 hours. Also SOEDE (1996) found that 18% animals presented RTH for 16 hours or less and many animals presented a discontinuous or very short symptomatology. Thus, considering the mentioned aspects, RTH should not be considered as an efficient procedure in the estrus detection.

3. Conclusion

The artificial intrauterine insemination (IUAI) allows for better use of the ejaculates, compared with the intracervical artificial insemination ICAI). This condition is possible, as taking into account that a lower spermatic concentration can be used in the insemination dose. The IUAI technique can be used at commercial farms in substitution to ICAI without endangering the reproductive efficiency.

In spite of the progresses and refinement of the different artificial insemination techniques, the deep intrauterine insemination (IUPAI) is a promising procedure. In this context, the possibility for using the insemination doses with small volume and reduced spermatic concentration will optimize the use of the males, as providing economical advantages to the farms. In addition, the use of the reduced volume and low spermatic concentration in IUPAI will allow progresses in the use of frozen semen.

However, the high cost of the pipette for this procedure and the difficulties in execution of the technique still represent impediments for its implantation in commercial farms. The results still inferior in the reproductive efficiency, when compared with ICAI and IUPAI, are added to those factors. Thus, it is evident that IUPAI is a technique with promising future to be used commercially at the farms, since the costs are decreased and the reproductive efficiency is not endangered.

The appropriate procedure of the artificial insemination is not the only factor determining the obtainment of desirable results in the reproductive efficiency. Thus, special attention must be to factors that can, direct or indirectly, influence the results.

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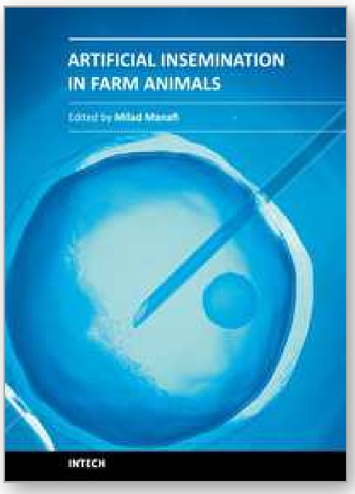

Artificial Insemination in Farm Animals

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Artificial insemination is used instead of natural mating for reproduction purposes and its chief priority is that the desirable characteristics of a bull or other male livestock animal can be passed on more quickly and to more progeny than if that animal is mated with females in a natural fashion. This book contains under one cover 16 chapters of concise, up-to-date information on artificial insemination in buffalos, ewes, pigs, swine, sheep, goats, pigs and dogs. Cryopreservation effect on sperm quality and fertility, new method and diagnostic test in semen analysis, management factors affecting fertility after cervical insemination, factors of non-infectious nature affecting the fertility, fatty acids effects on reproductive performance of ruminants, particularities of bovine artificial insemination, sperm preparation techniques and reproductive endocrinology diseases are described. This book will explain the advantages and disadvantages of using AI, the various methodologies used in different species, and how AI can be used to improve reproductive efficiency in farm animals.

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