Research Article

Survey of Artificial Insemination Practices in Algeria

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Abstract | A survey was conducted to describe the different practices of artificial insemination (AI). A total of 223 (55%) Algerian inseminators were surveyed. The questionnaire was divided into 4 groups: the first group involved general data about the inseminator (years of experience, number of AI performed yearly, % of AI achieved during natural heat, % of breeders who use AI); the second group focused on various practices employed before AI (confirmation of estrus, assessment of body condition); the third group examined practices applied during AI (thawing and semen deposition); the fourth and last group reviewed practices used after AI (monitoring the return to estrus and pregnancy diagnosis). One in two inseminators had more than five years of experience and 71% had achieved more than 200 annual AI which were influenced by the inseminator's years of experience ($p<0.001$). Sixty nine percent had achieved more than half of AI during natural heat 12 hours after the onset of the oestrus. Before thawing of semen, 43% had palpated the uterine tone and follicle, 65% had reported that less than 11% of females were not in heat. Thawing semen in warm-water (35-38˚C) for 40 seconds is the most commonly used practice. Before AI, only 36% used a protective plastic sheaths. Essentially the semen is deposited in the uterine body. Once the insemination gun has been removed, inseminators were more likely to massage the uterus than to stimulate the clitoris (91 vs. 19%). In the case of non-return to heat, 53% have diagnosed pregnancy through manual palpation rather than ultrasonography (73 vs. 27%). The frequency of pregnancy diagnosis was influenced by the number of breeders who practice AI ($p<0.05$), but not by the number of annual AI. The practice of AI was reported by the majority of respondents. Correct insemination practices will result in better fertility. Further training is required for the inseminators, if we want to use AI successfully to maintain a sustainable dairy industry in the future.

Keywords | Cattle, Artificial insemination, Practices, Fertility, Algeria

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INTRODUCTION

The Algerian milk production (3.1 billion litres) meets the needs of only 56% of the population (5.5 billion litres equivalent in milk) (National Inter Professional Office for Milk, ONIL 2012). Breeding cattle is one of the main priorities of the Algerian state in the area of agriculture. Since 1969, the Algerian state has organized the importation of pregnant dairy heifers, ensured the importation of

powdered milk (about 300,000 tons which amounts to 500 million dollars during these past years) and intensified the genetic improvement of bovine livestock by encouraging the use of artificial insemination (AI) through the creation of The National Centre for Artificial Insemination and Genetic Improvement (CNIAAG) in 1988. This institution ensures the production, processing and conservation of bovine semen, the popularization of AI and inseminator training. The CNIAAG manages the activity of 406 inseminators, who practice in more than 150 circuits across the national territory (CNIAAG, 2012). The number of AI increased from 50,000 in 2002 to more than 190,000 in 2012. AI penetration rates were 47% in the Central region, 33% in the East, and 20% in the West. AI essentially involves imported foreign breeds (Holstein, Montbeliarde, Fleckvieh and Brown Swiss). These breeds represent 30% of the national dairy bovine livestock, estimated to 966,000 cows and heifers (CNIAAG, 2012).

Despite concerted efforts, the average milk production (<15 litres per day) remains below expectations. One reason for this low milk production is extended calving intervals. Their calculated average value out of 1200 lactations is between 430 and 435 days (Bouzebda, 2007; Miroud, 2009). Infertility is probably partly responsible. Indeed the pregnancy rates at the time of the first insemination (AI1) ranges from 25 to 31% (Bouzebda, 2007; Miroud, 2009).

Diverse factors related to the animal, the breeder and the inseminator are responsible for this infertility. A weak AI success rate after placental retention or puerperal infection has been reported (Lopez-Gatius et al., 2005). The female's body condition score (BCS) must be taken into consideration in order to determine the appropriate timing of AI in postpartum. An insufficient BCS (< 2.5) at the time of insemination is a possible cause of a decline in the AI1 success rate (Loeffler et al., 1999).

AI practices include heat detection, timing of insemination, site of semen deposition and techniques. The details of this practice have been reported in several publications (Lopez-Gatius, 2000; Saacke, 2008; Lopez-Gatius, 2012).

Although the importance of confirming oestrus before insemination has been documented (Lopez-Gatius and Camon-Urgel, 1991), heat detection remains to be a major problem. According to Sturman et al. (2000), 19% of females were inseminated with a high level of milk progesterone. The timing of AI in relation to the onset of heat is equally essential. A significant difference in gestation rates (48.1%, 63.7% and 55.9%) was reported when AI was performed between 0 to 4 hours, 4 to 24 hours and more than 24 hours, respectively, from the onset of heat (Dorsey et al., 2011). That would indicate that the ideal timing for insemination is 12 to 18 hours after the onset of heat (Roelofs et al., 2005).

The process of thawing bovine semen can also have a significant effect on the fertility rate. A significant increase in the conception rate (35 vs. 27%) was reported when thawing was done in warm-water (33-35˚C) compared to air-thaw procedure (Dejarnette and Marshall, 2005). Thawing must occur at a temperature of 34˚C to 38˚C for a minimum of 20 seconds for a thin straw (0.25 ml) and 40 seconds for a medium straw (0.5 ml) (Dejarnette et al., 2004). Unfortunately, thawing the straw in a shirt pocket, air-thaw or even in an insemination gun are still widely practiced (Dejarnette et al., 2000).

Fertility depends on the inseminator. A highly significant difference in conception rate (45 vs. 27%) was observed between professional technicians and breeders who practice AI (Dalton et al., 2004). The use of a protective plastic sheath during AI can contribute to an improved conception rate. Bas et al. (2010) reported a significant increase in the gestation rate (42.7 vs. 36.1%) in females inseminated with protective plastic sheaths.

It has traditionally been recommended to inseminate the female in the uterine body. There was a declined by 22% in pregnancy rate when AI was performed in the cervix (Gwasdauskas et al., 1986). Senger et al. (1988) documented a highly significant increase in conception rate when semen was deposited in the uterine horn as opposed to the uterine body (64.6 vs. 44.7%). Nonetheless, deep-horn AI requires previous palpation of the ovaries in order to identify which horn (right or left) has the follicle (Lopez-Gatius and Camon-Urgel, 1988).

A study conducted by Loeffler et al. (1999) determined that the presence of transparent mucus on the

tip of the gun after AI was positively associated with an increased conception rate.

A reduction of 50 % was noted in the conception rate when an abnormal discharge (blood, pus) appeared on the gun following AI. Many authors have stressed the beneficial effect of lightly massaging the uterus and clitoris at the time of AI on conception rate. An increase in the success rate of AI1 of 18% (62 vs. 44%) was observed after 2 minutes of uterine massage (Hall and Plasto, 1970). Clitoral stimulation for 3 seconds at the time of AI increases the conception rate up to 15% at AI1 (74 vs. 59%) (Lunstra et al., 1985). This has been attributed to the positive effect on the secretion of oxytocin and LH (Randel et al., 1975). The lack of inseminator experience contributes to an increase in the time between thawing the straw and deposit in the uterine body. As such, this time was 4.2±0.17 min and 5.8±0.22 min, respectively, for technicians and inseminators/breeders (Dalton et al., 2004). The interval between thawing and insemination can be extended up to 15 minutes, if the straw can be maintained at ambient temperature and with strict hygiene during AI (Dejarnette et al., 2004).

The technique of inseminating is a skill requiring adequate knowledge and experience. Semen must be deposited within the tract of the cow at the best location and at the best time to obtain acceptable conception rates. Improper AI techniques can negate all other efforts to obtain conception. The present study aims to describe the different practices involved in performing AI, and to discuss these findings in order to make recommendations to Algerian inseminators.

MATERIALS AND METHODS

The survey consisted of 66 questions divided into 4 groups: the first group (24 questions) addressed general data about the inseminator (professional duties, age, sex, year and faculty where doctorate of veterinary surgeon was obtained, type of clinical activity, number of years of experience practicing AI, number of AI completed annually, region practiced in). The context of inseminator activity was characterised by the types and breeds of cattle involved, the size of the herd, the number of breeders among their clientele, the percentage of breeders who use AI, the percentage of AI achieved during natural heat as well as the material available to the inseminator (number of insemination guns, semen storage tanks, thermometers and thawing thermoses, protective plastic sheaths, vaginoscope, ultrasonograph).

The second group (12 questions) relates to various practices employed before performing AI (anamnesis, method of vaginal examination, factors that confirm oestrus, timing of AI, evaluation of BCS, BCS not recommended for AI, estimation of percentage of cows that are not truly in heat on the day of AI, as well as the method used to make that diagnosis).

The third group (17 questions) involves the practices applied during AI (monitoring level of liquid nitrogen in the canister, methods for thawing semen, wiping the straw, checking for the presence of sperm, use of a protective plastic sheath, method for cleaning the vulva, anatomic site of AI, pressure exerted on the gun, speed at which gun is withdrawn, sheath verification, duration of the AI, uterine stimulation and clitoral massage).

The fourth and last group (13 questions) relates to various practices after AI (filling the AI certificate, recording AI dates, monitoring return to heat, percentage of pregnancy diagnoses and method used manual versus ultrasonographic, confirming gestation stage, coding data, inseminator's views, and their ongoing training needs).

The study was conducted from January to September 2012 with a total 406 inseminators. The CNIAAG ensured the distribution of questionnaires through its three regional administrations (Central: Algiers, East: Setif, West: Oran) and medical practitioners. We emphasized to inseminators that to follow their usual AI practices in the field.

Statistical Analysis

Although the study was basically descriptive yet a statistical analysis was undertaken with the SAS statistical program following the PROC FREQ procedure (SAS 2001) using the Chi-square and Fisher's exact test.

RESULTS

General data

The survey's response rate was 55% (223/406). The inseminators who participated in the study were ei-

ther less than 31 years old (23%), between 31 and 40 years of age (42%) or over 40 years (35%). They have less than 6 years (48%) or more than 5 years (52%) of experience, respectively. Forty two percent of them have an exclusively rural practice and 58% of them participate in both rural and canine activity. For the most part (71%), they performed over 200 AI per year (36%, 200 to 400, and 35%, > 400 AI per year). The number of annual AI was significantly higher ($p<0.001$) when the inseminator's years of experience was high. In fact, 27.5% of inseminators with more than 5 years of experience performed more than 400 AI per year. On the other hand, 21.9% with less than 6 years of experience performed less than 200 AI per year (Table 1).

One in two inseminators has access to one AI gun, 44% have 2 guns. The majority own neither a vaginoscope (59%), nor a thermos (68%) for thawing semen, 68% have 1-2 thermometers. Many (78%) have two storage tanks (a big and a small). Only 27% have access to an ultrasonograph.

Seventy per cent of inseminators have a clientele that includes more than 50 breeding farms, out of which an average of 52% performed AI exclusively. The majority (69%) performed more than half of their AI on natural heat, approximately 12 hours after the onset of oestrus. In 2/3 of these cases AI involve imported livestock (Holstein and Montbeliarde) or livestock from local cross-breeding.

Practices applied before AI

The majority of inseminators systematically have achieved anamnesis before AI. This includes the date and type of calving, AI dates, date and signs of heat, the possible presence of postpartum pathologies. Eighty nine per cent of them controlled oestrus, usually before thawing the semen. This is accomplished through manual palpation of the genital tract and a vaginal examination. Forty-four percent of inseminators have confirmed oestrus by identifying the uterine tone, 43% by palpating the ovarian follicle and only 13% of them on uterine tone, the presence of a follicle and of a mucus discharge (Table 2). These different approaches in assessing oestrus have showed no significant relationship with the inseminator's years of experience or the annual number of AI.

After control of oestrus, many inseminators (65%) have estimated that less than 11% of females would not have been in true heat and consequently not ready for AI. For 25% of inseminators, this percentage would range between (11-20%). Very few of respondents (10%) recognized that more than 21% of females would not have been in heat (Table 2). Forty four per cent of inseminators have practiced transrectal palpation to identify a female who is not in heat, 35% used anamnesis and transrectal palpation and only 21% relied on anamnesis. The present study demonstrates that a high number of inseminators (92%) have evaluated the animal's BCS before its AI, 74% of them have estimated that a BCS ≤ 2 has a negative effect on fertility.

Practices applied at the time of AI

The majority (92%) of the inseminators monitored the level of liquid nitrogen before handling the straw. Eighty per cent of them thawed the straw in warm-water (35-38°C) for 40 seconds (Table 3). Once the straw has been thawed, it is usually wiped and the presence of sperm was verified. Few inseminators (36%) used a protective plastic sheath to avoid potential contamination of the straw in the genital tract. The majority (93%) cleaned the vulva before AI in order to avoid accidental penetration of faecal matter in the vagina or uterus via the gun sheath. In that case, 54% of them used paper towel, 28% tissue, 13% water, and 5% used antiseptic water.

Table 1: Effect of number of years of practice on the number of AI per year

Number AI per year	≤ 200	200-400	>400	Total
≤ 5 years (n= 105)	21.9^{a} (47)	19.5^{b} (42)	7.4^{c} (16)	48.8
>5 years (n=110)	7.4^{a} (16)	16.3^{b} (35)	27.5^{c} (59)	51.2
Total (n=215)	29.3	35.8	34.9	100

Data have different superscript letter within the same row were statistically different ($P<0.001$). The values in the parenthesis indicates the effective of inseminators.

Table 2: Frequency of responses of practices performed before AI

Method of diagnosis oestrus	**Uterin tone**	**Uterin tone/Follicle**	**Uterin tone/Follicle / Mucus**
Frequency of responses (n = 179)	44% (78)	43% (77)	13% (24)
% of females not heat at the time of AI	**≤10**	**11-20**	**>21**
Frequency of responses (n = 204)	65% (133)	25% (50)	10% (21)

Table 3: Frequency of responses of practices performed at the time of AI

Method of thawing	**Air**	**Water**	**Warm-Water**
Frequency of responses (n = 212)	10% (22)	10% (22)	80% (168)
Semen deposition	**Uterine body**	**Uterine horns**	**Both methods**
Frequency of responses (n = 218)	74% (161)	15% (33)	11% (24)

Table 4: Frequency of responses of practices performed after AI

Diagnosis pregnancy	**Manual**	**Ultrasonography**	**Both methods**
Frequency of responses (n = 214)	73% (156)	5% (10)	22% (48)
Period of manual diagnosis (days)	40-60	61-90	> 90
Frequency of responses (n = 205)	9% (19)	65% (134)	26% (52)
Period of ultra -sonographic diagnosis (days)	30-40	41-60	> 60
Frequency of responses (n =58)	55% (32)	45% (26)	0% (0)

When AI was performed by 74% of inseminators in the uterine body (Table 3), the majority of them pressed lightly on gun at the time of AI and withdraw it slowly in order to avoid harming the genital tract. Most of them (91%) performed a slight uterine massage rather than clitoral stimulation (19%). For 90% of them, AI was performed in less than 5 minutes.

Practices applied following AI

Following AI, many inseminators filled the AI certificate (73%), recorded the date of AI in the register (68%), and advised the breeders to monitor the return of heats (100%). In the event of non-return of heat, one in two inseminators diagnosed pregnancy. Pregnancy was diagnosed in 73%, 5% and 22% by manual palpation, ultrasonography, or both the methods, respectively. Manual diagnosis is done often (65%) during the 3rd month following AI. Ultrasonographic diagnosis also takes place 30 to 40 days after AI (55%), compared to 41 to 60 days after (45%) (Table 4). The gestational stage was confirmed in 95% of cases.

In 58% of the cases, inseminators who performed more than 200 AI per year used a computer to record data ($p<0.05$). Many of them (75%) recorded information relating to the parameters and pathologies of

the reproduction.

When asked if completing this survey motivated them to reflect on their AI practices, one out of two respondents entirely agreed, 13% moderately agreed, 25% moderately disagreed, and 12% disagreed entirely. This survey indicated that they would like to pursue training in the following areas: reproduction monitoring (26%), postpartum pathologies (18%), ultrasonography (22%), nutrition (18%) and mammary health (16%).

DISCUSSION

This survey of 223 inseminators revealed that 69% of them practiced AI during natural heat 12 hours after the onset of oestrus, which should be normally observed by the breeder. The conception rate can be maximized by choosing the right time for AI in relation to onset of oestrus. Approximately 12 hours after the onset of oestrus is therefore considered optimal timing for AI (Saacke, 2008). The traditional AM/PM, PM/AM rule: cows first seen in standing heat in the morning (AM) would be inseminated in the afternoon (PM) and those observed standing in the evening (PM) would be bred in the next morning (AM), remains the most effective way in farms with best oestrus detection (DeJarnette et al., 2004).

The majority (92%) take the estimated BCS into consideration at the time of AI. Three quarters of inseminators estimated that a BCS ≤ 2 has a negative effect on fertility. Several authors have reported the negative effect of an inadequate BCS (< 2.5) at the time of AI on the success of AI1 or the pregnancy rate (Pryce et al., 2001). According to Loeffler et al. (1999), a BCS of 3 at the time of AI ensures a better pregnancy rate. Most inseminators (89%) have examined the reproductive tract at the time of AI in order to ensure that they inseminate cows when they are truly in heat and exclude those who are not ready for AI. A precise manual diagnosis of oestrus can be made following a transrectal examination of the reproductive tract (Lopez-Gatius and Camon-Urgel, 1991). According to Lopez-Gatius (2011) follicular palpation is closely related to the success of AI. Forty-three percent of the inseminators responded that they confirm oestrus by palpating the uterine tone and the follicle, which are both considered to be significant indicators of successful AI (Loeffler et al., 1999). A diagnosis of ovarian follicles greater than 10 mm in diameter is more precise if it is done through ultrasonographic rather than manual palpation (Pieterse et al., 1990). The manual palpation of follicles<10 mm can be inaccurate and depends on the overall size of the ovary, the degree of relaxation of the rectal wall, BCS, and competence of the examiner (Hanzen et al., 2000). It is important to note that many inseminators are inadequately trained to examine the genital tract and ovaries despite the significant number of transrectal palpations practiced daily (Lopez-Gatius, 2000). The survey revealed that after control of oestrus, 65% of Inseminators respondents estimated that less than 11% of females were not in heat, and a quarter of them estimated that percentage was between (11-20%). Various rates of inseminated females with a high level of milk progesterone have been reported such as 19% (Sturman et al., 2000) and 40% (Nebel et al., 1987).

Thawing semen in warm-water (35-38°C) for 40 seconds is the most commonly used thawing procedure reported by our inseminators (80%). A significant increase in the conception rate (35 vs. 27%) was reported when thawing occurred in warm-water (33-35°C) as opposed to air (Dejarnette and Marshall, 2005). Kaproth et al. (2005) also reported a significant increase (66.1 vs. 62.4%) in the fertility rate when thawing is done in warm-water 35°C for 30 seconds compared to pocket-thaw.

Few of those surveyed (36%) used a protective plastic sheaths as a preventive measure against possible contamination of the genital tract. A study conducted by Bas et al. (2010) concluded that using a protective sheath significantly increases the pregnancy rate (42.7 vs. 36.1%) and furthermore reduces contamination of the AI gun by 40% (61.53 vs. 100%) compared to the control group. On the other hand, King et al. (1984) concluded that the use of a protective sheath does not increase AI conception rates.

This study revealed that inseminators preferred depositing semen in the uterine body as opposed to the uterine horns (74 vs. 15%). This is due in part to the training they received from CNIAAG, which has through its teaching traditionally promoted the uterine body as the ideal insemination site. Several studies have reported significant increases in conception rates during a cornual inseminations compared to the uterine body, such as (64.6 vs. 44.7%, Senger et al.,

1988) and (30 vs. 19%, Mc Kenna et al., 1990). In contrast, no significant difference in conception rates between cornual inseminations and those performed in the uterine body (Momont et al., 1989). Nevertheless, specialized training in the technique of deep AI is necessary (Lopez-Gatius, 2000).

The majority of inseminators massage the uterus following AI instead of stimulating the clitoris (91 vs. 19%) in an effort to stimulate contractions of the uterus and encourage sperm to move along the genital tract. A study conducted by Lunstra et al. (1985) showed that clitoral stimulation following AI increases the pregnancy rate of 15%. Bozkurt et al. (2007) reported that clitoral stimulation has no effect on the pregnancy rate.

Insemination usually takes place in less than 5 minutes. This duration has no significant relationship with the number of annual AI ($p>0.05$). Time duration between thawing and semen deposition (4.2±0.17vs.5.8±0.22min) was reported by technicians and inseminators/breeders, respectively (Dalton et al., 2004). A maximum period of 10 to 15 min between thawing and semen deposition would be acceptable in terms of fertility on condition that the straw be kept at an ambient temperature (35°C) and that strict hygiene be maintained during AI (Dejarnette et al., 2004).

Many of the inseminators confirmed pregnancy in more than half of the inseminated livestock, usually by manual palpation. However, this inexpensive method can only be used after the 6th, even 9th week of gestation (Hanzen et al., 1991). Ultrasonographic confirmation is both an early (diagnosis from 25th day of gestation) and a reliable diagnosis because it distinguishes between animals that are pregnant (sensitivity 90%) from non-pregnant (specificity 80%) with an 87% degree of accuracy (Hanzen et al., 1993). A high frequency in pregnancy diagnosis was recorded by breeders who practice AI ($p<0.05$), undoubtedly due to the financial aid granted by the Algerian state. This profitable financial aid is given to dairy breeders as part of a national agricultural development plan (PNDA), initiated in 2000, in order to promote AI in Algeria.

Various AI practices can considerably affect a female's fertility. Inseminators seem to follow the second half of heat, which is the ideal time for AI. Examining the oestrus state remains an important procedure usually practiced by our inseminators before each AI, but the sound practice of transrectal palpation as a means of confirmation (the uterine tone, follicular palpation) was not readily taken into consideration. Procedures relating to the thawing process, semen deposition, uterine massage, as well as duration of the AI were widely followed. Despite their numerous advantages, the protective plastic sheaths, cornual AI and clitoral stimulation following AI were rarely used. Unfortunately, despite the possibility of early diagnosis of pregnancy through ultrasonography, many inseminators rely on manual confirmation of pregnancy.

After considering these results, we recommend the following: i) training for dairy breeders on detection means and identification of characteristics of an animal in heat. ii) Retraining and awareness sessions for inseminators regarding transrectral examination of the genital tract and deep AI. iii) Use of protective sheaths as a preventive measure. iv) Slightly massaging the clitoris after each AI. v) Using ultrasonography to diagnose early pregnancy and assess fertility potential in dairy cattle.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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