

**STUDY ON QUALITY OF FROZEN SEMEN AND
EFFICIENCY OF AI TECHNICIANS AND THEIR
IMPACT ON REPRODUCTIVE PERFORMANCE OF
BUFFALOES UNDER AI NETWORK PROGRAMME**

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B.V.Sc. & A.H.



**DEPARTMENT OF ANIMAL REPRODUCTION GYNAECOLOGY AND
OBSTETRICS
POSTGRADUATE INSTITUTE OF VETERINARY EDUCATION AND
RESEARCH
KAMDHENU UNIVERSITY, GANDHINAGAR-382010**

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UNDER AI NETWORK PROGRAMME**

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DEDICATED

TO

ALMIGHTY,

PARENTS

and

FRIENDS

ABSTRACT

**STUDY ON QUALITY OF FROZEN SEMEN AND EFFICIENCY OF AI
TECHNICIANS AND THEIR IMPACT ON REPRODUCTIVE PERFORMANCE
OF BUFFALOES UNDER AI NETWORK PROGRAMME**

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ABSTRACT

The research entitled “Study on Quality of Frozen Semen and Efficiency of AI technicians and their impact on Reproductive Performance of Buffaloes under AI Network Programme” was conducted to know the efficiency of AI technicians and reproductive performance of buffaloes under AI network programme as well as to evaluate the post thaw motility of spermatozoa by CASA.

The information on attributes of 83 AI technicians were collected which revealed that highest percentage (38.6%) of the AI technicians were from 31 to 40 years of age group. The percentage of AI technicians obtained higher secondary, graduation and above level of education were 38.6 and 36.1, respectively. Seventy seven per cent of AI technicians had undergone basic as well as refresher type of trainings which has contributed towards success of AI. All the AI technicians have detected estrus prior to AI and all of them have observed clear mucus discharge in estrus animals. Seventy four per cent of the AITs have confirmed detection of heat by palpation of ovarian follicle. Ninety four per cent of the AITs have practiced correct site of semen deposition.

Fifty per cent of the AI technicians have performed insemination at the ideal time which resulted in significantly higher conception rate (46.18 ± 0.97) in buffaloes of Sabarkantha district followed by Arvali (41.02 ± 0.99) and Panchmahal (40.66 ± 0.99). Fifty three per cent of the AI technicians have followed standard thawing temperature and time (37°C and 30 seconds).

Sixty two frozen mini straws randomly selected from 31 AI technicians were analysed for post-thaw motility, different velocities and sperm abnormalities. The overall

mean values of velocity ($\mu\text{m/s}$) of post-thawed spermatozoa of samples were: Average Path Velocity (VAP) 100.86 ± 1.97 , straightline velocity (VSL) 86.70 ± 1.76 and curvilinear velocity (VCL) 157.10 ± 3.13 . Further, the mean values of other velocity parameters of post-thawed sperms of samples were: ALH $5.98 \pm 0.13 \mu\text{m}$, BCF $35.53 \pm 0.34 \text{ Hz}$, Straightness 82.66 ± 0.44 per cent, Linearity 55.48 ± 1.42 per cent, Elongation 56.23 ± 0.05 per cent and Area $25.86 \pm 0.43 \mu\text{m}^2$.

Straightness (STR) of the spermatozoa was found significantly higher ($p < 0.01$) in education group I, followed by groups II, IV and III. Similarly linearity (LIN) was found significantly higher in group I followed by groups II, IV and III. The linear motility (59.88 ± 2.02) and wobbling motility (68.49 ± 1.45) were significantly higher in group IV than those of groups I, II and III.

Highly significant difference was observed in the coiled tail characters of frozen-thawed spermatozoa in experienced groups of AI technicians. The difference in group I and II were significantly lower than groups IV, III and V. Similarly the coiled tail count was significantly higher in group IV followed by groups I, II, III and V.

Motile mean elongation, slow concentration, slow count, slow sample, total concentration, total count and total sample were found significantly higher from the samples collected from AITs who received basic training. The corresponding overall mean post-thaw motility and progressive motility percentage of the above profile parameters were 73.59 ± 13.57 and 50.67 ± 1.52 ; 73.58 ± 13.57 and 50.67 ± 11.99 ; 73.59 ± 13.57 and 50.67 ± 11.99 ; 73.58 ± 13.57 and 50.67 ± 11.94 ; 73.59 ± 13.57 and 50.67 ± 11.99 , respectively.

Age at first AI (months) in buffaloes of Sabarkantha district was lower than the age at first AI for Arvalli and Panchmahal districts. Similarly the age at first calving (AFC) in buffaloes of Sabarkantha district was lower than the buffaloes of Arvalli and Panchmahal districts.

Present work on selected AI technicians' profile and their employed practices had an influence on AI success rates. Some of these positive influences of attributes have showed influence on post-thaw motility and velocity parameters assessed by CASA. There is a dire need to take up further studies in such buffaloes to correlate CASA traits of fresh and frozen thawed semen with sperm motility, live sperm percentage and reproductive performance of the buffaloes.

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CERTIFICATE -I

Date: / /2018

This is to certify that **Kodiyatar Madhuri Dilipkumar (Reg No-2015010060021002)** has successfully completed the comprehensive examination of Major and Minor subject held on **14/03/2017** as required under the regulation for ~~M.V.Sc./M.Tech/~~ ~~M.F.Sc./Ph.D.~~ in Veterinary Sciences & Animal Husbandry/~~Dairy Technology/ Fisheries~~ Sciences.

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CERTIFICATE -II

Date: / /2018

This is to certify that the thesis entitled "**Study on quality of frozen semen and efficiency of AI technicians and their impact on reproductive performance of buffaloes under AI network programme**" submitted for the degree of M.V.Sc. in Veterinary Sciences & Animal Husbandry / ~~Dairy Technology/ Fisheries Sciences~~ in the subject of **Animal Reproduction, Gynaecology and Obstetrics** embodies bonafide research work carried out by **Kodiyatar Madhuri Dilipkumar** under my guidance and supervision and that no part of this thesis or research work has been submitted for any other degree. The assistance, guidance and help received during the course of investigation have been fully acknowledged. The draft of the thesis was also approved by the advisory committee on **12 /10 /2017**.

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Date: / /2018

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DECLARATION

This is to certify that the whole of the research work reported in this thesis in partial fulfillment of requirement for the award of the degree of M.V.Sc. in Veterinary Sciences & Animal Husbandry in the subject of **Study on quality of frozen semen and efficiency of AI technicians and their impact on reproductive performance of buffaloes under AI network programme** is the result of investigations done by undersigned under the direct guidance and supervision of **Dr. R. G. Shah (Associate Director of Research)**, Kamdhenu University, Gandhinagar and no part of the research work has been submitted for any other degree so far.

Place: Gandhinagar

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Place: Gandhinagar

Kodiyatar Madhuri D.

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ABBREVIATIONS

%	Percent
±	Plus or minus
°C	Degree Celsius
°F	Degree Fahrenheit
µm	Micro meter
µm/s	Micrometer per second
µm²	Square micro meter
µl	Microliter
AC	Alternating Current
AFC	Age at First Calving
AI	Artificial insemination
AITs	AI technicians
ALH	Amplitude of Lateral Head
ANOVA	Analysis of Variance
ARE	Sperm Area
BCF	Beat Cross Frequency
BCS	Body Condition Score
CASA	Computer Assisted Semen Analyzer
CR	Conception Rate
CL	Corpus Luteum
DC	Direct Current
DAHD	Department of Animal Husbandry, Dairying and Fisheries, Govt. of India
DVI	Digital Visual Interface
ELG	Elongation
<i>et al.</i>	And others
E₂	Estradiol
FAO	Food and Agricultural Organization
FSH	Follicle Stimulating Hormone
GB	Gigabyte
GnRH	Gonadotropin Releasing Hormone

Hrs	Hours
Hz	Hertz
ICAR	Indian Council of Agricultural Research
INAPH	Information Network on Animal Production and Health
kg	kilogram
LH	Luteinizing Hormone
LIN	Linearity
LN₂	Liquid Nitrogen
NDDB	National Dairy Development Board
pH	Negative logarithm of hydrogen ion concentration
PCR	Polymerase Chain Reaction
P₄	Progesterone
RAID	Redundant Array of Independent Disks
RPM	Revolutions per minute
SAG	Sabarmati Ashram Gaushala
SE	Standard Error
SOP	Standard Operating Procedures
SPSS	Statistical Package for the Social Sciences
STR	Straightness
TBS	Tris-buffered Saline
USB	Universal Serial Bus
VAP	Average Path Velocity
VCL	Curvilinear Velocity
VSL	Straightline Velocity
<i>viz.</i>	Videlicet (Namely)
<i>vs</i>	Versus
<i>vis-a-vis</i>	in relation to
<i>i.e</i>	That is
>	Greater than
<	Less than
=	Is equal to

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INTRODUCTION

Livestock is an important component of the Indian rural economy, where buffaloes play a prominent role in rural livestock production providing the milk, meat and work/draft force. Today, India is the top milk producer in the world as far as gross productivity is concerned, but per animal productivity is low. This is mainly because of poor genetic potential and nutritional status of indigenous animals. There is enough scope for the improvement of indigenous livestock through upgrading or crossbreeding and by applying various scientific approaches. Artificial insemination is one of them, which is the first generation biotechnological advancement that has made a profound contribution to the genetic improvement of livestock.

Buffaloes (*Bubalus bubalis*) are the major contributors of milk production in India. Approximately 51.06% of total milk production in India is from buffaloes (DAHD, 2014-15). Therefore, it plays a pivotal role in livestock sector of this country. In India, total buffalo population is approximately 21.23% of the total livestock population (19th Livestock census, 2012). Almost all the major riverine buffalo breeds are native of Indian subcontinent. Today, India is the only nation having substantial and varied biodiversity of buffalo germplasm, which includes about a dozen well defined breeds and a vast population of non-descript and low producer buffaloes.

Farmers prefer buffalo husbandry because high milk fat fetches good price, more disease resistance, efficient utilizer of feed and fodder, low maintenance etc. However there are some limitations such as silent estrus, seasonal breeder/summer anestrus, long pubertal age; some of which can be corrected by the improved managerial practices. The economic viability of a dairy herd depends upon normal

reproduction. Reproduction traits are affected by various factors such as nutrition, physiological status, season, heredity, infection, management, etc.

Artificial Insemination (AI) is not merely a novel method of impregnation in females, but, it is also a powerful tool being employed for livestock improvement. In AI, the germplasm of the males of superior quality can be effectively utilized with the least regard for their location in faraway places. By adoption of AI, there has been a considerable reduction in sexually transmitted diseases in animals.

It is known that the knowledge skill of AI technician is fundamental for the efficiency of the AI technique and that the lack of skill of this technician can limit the expected results in terms of higher conception rates, increase in milk production, reduction in sexually transmitted diseases and decreasing of service period and intercalving period.

For implementation of any breeding programme successfully, it is essential to have an effective network of AI services in the field condition. Under such AI network programme, field AI technicians perform insemination in cattle and buffaloes. The conception rate of the animals under the network varies as per the skill and efficiency of AI technicians. The quality of frozen semen at various AI centers and their impact on the reproductive efficiency of buffaloes are very important for the economy of dairy farmers. In India, where buffaloes are the most valuable livestock species, research on buffalo specific artificial breeding technologies and adoption of AI by buffalo owners are widely acknowledged. Resultantly, average milk yield of buffaloes in India increased from 3.4 kg in 1992 to 1993 to 4.57 kg/day/buffalo in 2009 to 2010.

In the new millennium, breeding projects such as the National Project for Cattle and Buffalo Breeding and the National Dairy Plan were initiated with focus on

genetic upgradation of bovine and buffalo population through streamlining AI services and support system in the country. Artificial Insemination started in India in the year 1939 using diluted liquid semen, and the frozen semen was introduced during late 1960s. During the year 2011, India produced 63 million bovine frozen semen straws including over one million buffalo semen straws through 49 semen stations. Artificial insemination services are provided through 71,341 AI stations numbering 52 million inseminations with overall conception rate of 35% in bovine and buffalo population. Research is also being conducted for improved AI conception rates with synchronization programmes and improved frozen-thawed semen quality, and the success rates in buffaloes are at par with AI in cattle.

Failure of fertilization and embryonic mortality, particularly after AI, has long been recognized as potential sources of loss in breeding females (Bas *et al.*, 1996). However, some studies have reported that impaired sperm quality leads to a lower percentage of embryos that develop to blastocysts (Shoukir *et al.*, 1998), poor blastocyst quality (Zhang *et al.*, 1998) and relatively high rates of pregnancy failure (Sanchez *et al.*, 1996). Saacke *et al.* (2000) suggested that failure in fertilization and subsequent embryonic development are of seminal origin. In line with this study, Zhang *et al.* (1998) reported that the factors with the highest predictive index for success within *in vitro* fertilization procedures were sperm motility, morphology, and the percentage of sperm cells with intact acrosomes.

Assessment of sperm motility is usually done subjectively under phase contrast microscope. The results largely depend on experience of technicians, thus implying great variation between laboratories making poor estimations of fertility (Rodriguez-Martinez, 2003). The variations in motility were 30-60% in subjective assessment of buffalo semen in the same ejaculates (Pant *et al.*, 2003 and Koonjaenak

et al., 2007). In order to decrease this variation, computer-assisted semen analysis (CASA) instruments were developed with software to analyze and record every sperm characteristic that improved the semen evaluation. The advantage is that they are considered to be more 'objective' and not only determine the proportion of motile spermatozoa, but also assess the kinetics of individual sperm (Mandal *et al.*, 2003). Furthermore, few scattered reports are available to know the quantitative evaluation of motility parameters of spermatozoa during different stages of cryopreservation.

Male fertility is an important factor in bovine reproduction, considering that the semen of any particular bull will be used for the insemination of several females. Fertility evaluation of males is primarily based on quality assessment of semen using conventional parameters such as morphology, concentration and motility of spermatozoa. The correlation between semen quality and fertility has been well documented (Kathiravan *et al.*, 2008; Freour *et al.*, 2012). Sperm motility is undoubtedly essential for fertilisation both *in vivo* and *in vitro*. Motility is indispensable for successful sperm transport, a step that can partly be bypassed by *in vitro* fertilisation. Thus, examination and determination of sperm motility is a significant part of semen quality evaluation since it is commonly regarded as one of the most important characteristics associated with the fertilising ability of spermatozoa.

Computer assisted sperm analyzer (CASA) is potentially a very powerful research and clinical tool. It refers to an automated system (hardware and software) to visualize and digitalize successive images of sperm, process and analyze the information and provide accurate, precise and meaningful information on the kinematics of individual cells. It also provides information regarding population summary statistics, *i.e.* mean values. Early systems required operator intervention, but

preferred systems would require the operator only to ensure that the system is functioning properly, to place the sample into the instrument and to examine/store output data. Underlying concepts of CASA have been illustrated by (Boyers *et al.*, 1989).

Application of AI with frozen-thawed semen using commercial extenders has been reported on a limited scale in buffalo. Moreover, the freezability and fertility of frozen-thawed buffalo spermatozoa are reported to be poor when compared with cattle spermatozoa (Kumaresan *et al.*, 2005). Hence, successful cryopreservation of bubaline semen using newer diluents devoid of egg yolk would aid in long-term storage of male gametes and the maintenance of genetic stock that could improve milk production and its associated economic value.

The effect of storage on frozen semen straws and handling of the containers varies among the AI technicians working under AI network programme. The initial motility and live sperm percent are correlated with CASA measured traits of fresh and frozen thawed semen. So the post-thaw motility of frozen semen by CASA and its relation to fertilization ability need to be determined. Evaluation of bovine sperm motility is necessary and the optimal post-thaw motility and viability of sperms in semen also contributes to the conception rate in field condition. The conception rates and efficiency of AI technicians are presumed to be factors influencing the AI success rate in the field condition under AI network programme in the buffaloes. It would be worthwhile to assess these factors and evaluate the frozen semen doses being used by AI technicians in the field condition. Therefore, a study has been planned with following objectives:

1. To study the efficiency of AI technicians under AI network programme in the field
2. To study the reproductive performance of the buffaloes under AI network programme
3. To study the post thaw motility of spermatozoa by Computer Assisted Sperm Analysis
4. To suggest remedial measures to improve the success rate of AI in field

REVIEW
OF
LITERATURE

The present investigation on reproduction of graded Murrah buffaloes, attributes of AI technicians and evaluation of motion characteristics of frozen-thawed sperms using CASA was made in three districts of Gujarat.

The pertinent literature on these aspects has been reviewed under the following sub-heads:

2.1 Reproduction in domestic buffalo

2.1.1 Puberty and age at first calving

2.1.2 Ovarian activity (CL/follicle)

2.2 Signs of estrus detection for AI

2.2.1 Estrus and estrus signs

2.2.2 Estrus behaviour

2.2.3 Service period

2.3 Artificial Insemination (AI)

2.3.1 Site of semen deposition

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2.3.3 Calving interval

2.4 Skills and knowledge of AI technicians

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2.5.2 Experience as an AI technician and performance of skills

2.5.3 Training received and performance of skills

2.6 Seminal characteristics and cryopreservation of semen straws

2.7 Semen evaluation

2.7.1 Computer assisted semen analysis

2.7.2 Initial and post-thaw motility of bovine semen

2.1 Reproduction in Domestic Buffalo

2.1.1 Puberty and age at first calving

The age at which estrus is first detected is referred to as puberty. Buffalo heifers attain puberty at about 24-30 months of age and at 225-275 kg body weight, *i.e.* when animals attain 55–60% of their adult body weight (Jainudeen *et al.*, 1993).

According to Jainudeen and Hafez (1993) river buffaloes exhibit first estrus at 15-18 months of age. The studies of different researchers provide proofs for significant variations in the time of puberty onset from 9.9 months in Egyptian buffalo heifers (Mohamed *et al.*, 1980), 18 months of age in Brazilian buffaloes (Okuda *et al.*, 1999), 20.7 months in Mediterranean Italian buffaloes and upto 45.5 months in the Surti buffaloes (Sule *et al.*, 2001). According to Peeva *et al.* (1993) the onset and course of puberty in buffalo heifers are not completely elucidated as available data are mostly based on visual observations without taking consideration for the endocrinological status of animals.

RIA blood progesterone (P₄) analysis in Murrah and its crosses with Bulgarian buffaloes indicate 19.9 months of age for puberty onset. Kanchev *et al.* (2010) affirmed that the puberty in Murrah buffaloes begins at 14.4 months of age, while in primitive breeds at 35.6 months of age. Baychev (2008) discussed the effect of season of birth of buffalo heifers on puberty onset and reported that animals born in the spring attained puberty at 404.3 ± 3.9 days, while those born in the autumn at 543.2 ± 19.5 days.

In the major part of cases, the onset of puberty is determined retrospectively on the basis of first calving date (Borghese and Mazzi, 2005), which does not always indicate accurately the exact time of the first estrus. Singh *et al.* (2010) reported that some buffalo heifers could remain anestrous for a long time, even when they have attained the puberty age for the breed, if the first estrus is anovulatory or the subsequent follicular development is not adequate.

The age of puberty in buffalo is 36 to 42 months in India (Borghese, 2003).

The age at first calving is 1925 ± 196 (days) in Jafrabadi buffaloes (Trivedi, 2000 and Sethi, 2003), while age at first calving in Bhadawari buffaloes is 48.6 ± 0.58 (months).

Table 2.1: Age at puberty and age at first calving in buffalo heifers as reported

Breed	Age at puberty (months)	Age at first calving (months)	References
Murrah	16-40	37-57	Rakshe, 2003
Murrah and crossbred	24-30	37-86	Sompala, 2002
Surti	30-36	33-56	Sethi, 2003
Bhadawari	28-32	48-51	Singh, 2000a; Sethi, 2003
Mehsana	-	46-47	Taneja <i>et al.</i> , 1999
Nagpuri	42-48	-	Kaikini, 1969
Nili-Ravi	23-36	40-42	Khanum <i>et al.</i> , 2012

The buffalo (*Bubalus bubalis*) population in the world is about 168 million heads: 161 million can be found in Asia (95.83 per cent); 3.7 million are in Africa, almost entirely in Egypt (2.24 per cent); 3.3 million (1.96 per cent) in South America, 40,000 in Australia (0.02 percent) and 5, 00,000 in Europe (0.30 per cent).

The *Bubalus bubalis* belongs to the class Mammalia, subclass Ungulata, order Artiodactyla, suborder Ruminantia, family Bovidae, subfamily Bovinae, tribe Bovini, which includes the following three groups: Bovina (cattle), Bubalina and Syncerina. Syncerina includes only the species *Syncerus caffer* (the African buffalo). Bubalina (the Asian buffalo) includes three species: *Bubalus depressicornis* or Anoa which

lives in Indonesia, *Bubalus mindorensis* which lives in the Philippines and *Bubalus bubalis* deriving from the domestication of the *Bubalus arnee*, the Indian wild buffalo.

The two sub-species (swamp and river) are inter-fertile and produce progeny with 49 chromosomes. Male crossbred progeny has sometimes displayed fertility problems while female progeny has manifested longer calving intervals only in the case of further backcross.

Reproduction in the buffaloes appears to be affected with delayed puberty, poor estrus expression, prolonged calving intervals and seasonal breeding limiting the reproductive performance. Females are known to have a smaller number of primordial follicles in their ovaries (Smith, 1990), a silent estrus (Kanai, 1983 and Bodhipaksha, 1987), a longer gestation period of 330 days (Manson, 1994) and a higher age at first calving (Moolmuang *et al.*, 2007); whereas buffalo bulls with a higher age at puberty (Chantarapateep, 1987 and McCool, 1989) and extremely low sperm concentration (Cuong, 1983 and Chantarapateep, 1987).

Reaching puberty is more related to body weight than to age. However, individual's genotype, nutrition, management, season of birth, climatic factors, occurrence of disease and the presence or absence of a mature male can influence the age at puberty (Bhatti *et al.*, 2007).

Buffalo heifers are slower to reach puberty as compared to cattle (Campanile *et al.*, 2010). The age at puberty is difficult to establish because of difficulties in estrus detection in this species and most estimations appear to have been extrapolated from the age at first calving (Barile, 2005).

Many factors influence the age at puberty in buffalo such as breed, season, climate, nutrition and growth rate (Hassan, 2005; Drost, 2007; Campanile *et al.*, 2010).

The reasons for the delayed onset of puberty in pre-pubertal buffalo heifers are only partly explained on the basis of low profiles of circulating thyroid hormones (Ghuman *et al.*, 2011 and Ingole *et al.*, 2012) low body fat (Ghuman *et al.*, 2011) or the inherently suboptimal functioning of the hypothalamo-hypophyseal-gonadal axis and the consequently low circulating hormones (Mondal *et al.*, 2007).

A close association between growth hormone and LH has been found with regards to attainment of puberty (Saini *et al.*, 1998). The highest LH concentrations were recorded just 1 month before puberty (Saini *et al.*, 1998). Pubertal heifers had follicular growth similar to adult buffaloes, however, growth rates were slower and the size of the dominant follicle was smaller in heifers (Presicce, 2004).

There is a wide variation in the age at puberty and age at first calving in buffalo heifers of different breeds. The age at first calving is influenced by many variables with India being highest in rural buffaloes.

The luteal phase of buffaloes lasts 15-16 days, and is characterized by the presence of a CL and elevated progesterone plasma levels, while the follicular phase lasts 4-6 days and is characterized by the absence of a CL, very low plasma progesterone levels and high plasma estrogen levels. Estrus activity in buffaloes is characterized by a marked seasonal influence. The duration of estrus is shorter, estrus expression is less marked during the low breeding season from May to July (summer or dry season) and this may be mistaken for anestrus. Mucus secreted from the cervix during estrus is less copious; the vulvar changes and expression of estrus mucus are less pronounced than in cattle (Presicce, 2007).

The animals without vaginal discharge may also be in estrus. In most buffaloes, the discharge is observed during palpation of the genital organs per rectum before AI. The discharge can be found on the tail, as well as on the gluteal region. In

buffaloes, the discharged mucus should be observed carefully in early hours of the morning or in late hours of the evening when the animal is in recumbent position.

Irrespective of breeding or non-breeding season, buffaloes show less intense expression of estrus compared with cattle (Ohashi, 1994) and also less estrus-associated mounting behaviour (Roy & Prakash, 2009).

The gestation length reported in river buffalo is around 315 days and in Zebu and Red Sindhi cattle 285-292 days (El-Fouly *et al.*, 2006). The gestation length of Murrah buffalo breed in Philippines is 314-317 days. The gestation length in buffaloes in Assam, India was 324.40 ± 0.21 days and was affected by the season of calving and gender of calf; buffaloes carrying male fetuses and calving during the breeding season had a significantly longer gestation period (Zaman *et al.*, 2007). The gestation length for buffaloes varied from 320-345 days (Cuong, 1983).

The maximum growth of fetus was recorded during 301-330 days of gestation (Van Hanh *et al.*, 2013).

The Carabao buffalo in Philippines had an average age at first calving of 43 months, slightly shorter than river buffalo (45- 52 months) and considerably longer than the Red Sindhi cattle (35 months). The age at first calving for the Murrah x Carabao crossbreed females was recorded at 39 months, which is shorter than for both purebreds (Karen *et al.*, 2007).

The calving interval of around 500 days has been reported in buffaloes. Animals with a calving interval of less than 500 days were considered as with a short calving interval (Moolmuang *et al.*, 2007).

The interval between the onset of estrous and the LH surge can vary from 1 to 12 h in buffaloes (Seren *et al.*, 1995) and ovulation occurs between 26 and 33 h after the LH surge (Seren *et al.*, 1995; Porto-Filho *et al.*, 1999).

Another peculiar feature of buffalo reproduction is the high incidence of double ovulations. Interestingly, it has been reported that only 0.06% of double ovulation leads to twin pregnancies. However, the occurrence of double ovulations reduces the efficiency of AI only in case of spontaneous estruses, but not in case of induced estruses (Zicarelli *et al.*, 1997).

Noakes *et al.* (2001) reported that buffalo is characterized by delayed puberty, prolonged post-partum ovarian inactivity, long intercalving intervals and a tendency for seasonality.

Buffaloes become increasingly seasonally polyestrous with distance from the equator (Baruselli *et al.*, 2001a; Campanile *et al.*, 2010).

Buffaloes that calve during the non-breeding season have an extended postpartum anestrous period with a proportion not resuming ovulation until the following breeding season (Zicarelli, 2007).

The influence of photoperiod means that, without intervention, buffaloes have seasonal cycles in conception, calving and milk production (Campanile *et al.*, 2010).

The need to make calving and weaning coincide with both the most suitable season, in order to satisfy the heat and nutritive requirements of the offspring, and with the period in which the causal agents of infections and infestations express less pathogenic effect (Zicarelli *et al.*, 1997) represents one of the causes which gave rise to this adaptation process.

The individuals born in more favourable conditions have given rise to natural selection of subjects with a seasonal reproductive behaviour better suited to the survival of the species, whose reproductive features were probably determined by stimuli incorporated in the central nervous system during gestation or during the first days of life (Zicarelli *et al.*, 1997).

2.1.2 Ovarian activity (CL/follicle)

The absence of large follicles and CL on the ovaries is sign of true anestrus. Palpation and direct observation of ovaries collected from slaughtered anestrus swamp buffaloes showed flat, solid ovaries which were significantly reduced in size.

Chantarakhana *et al.* (1981) studied the onset of ovarian activity during the postpartum period by ovarian palpation per rectum, expression of estrus, milk or plasma hormone profiles. There is an extremely high variability in the duration of postpartum period in the buffaloes. The first estrus can appear between 51.6 ± 15.3 to 144 ± 22 days after calving, depending on the region and breeding conditions. It has been highlighted the effect of the season on follicular population and oocyte developmental competence. Although the follicular population and the number of oocytes recovered per ovary is not influenced, a significant increase of both cleavage (71.7 vs 58 per cent) and blastocyst (26.5 vs 18.8 per cent) rates is observed during autumn months compared to spring months, indicating a seasonal effect on oocyte developmental competence, that reflects the *in vivo* reproductive pattern (Di Francesco *et al.*, 2011).

No follicular activity was present in buffaloes during the first 30 days after calving. The results of plasma progesterone determination indicated that the first postpartum ovulation occurred beyond 60-90 days in river buffaloes (Sethi, 2003; FAO, 2003).

Under optimal conditions, the regression of the CL of pregnancy is very rapid, with a complete regression by 7 ± 2 days from calving and by day 10; the postpartum CL is palpable as a small hard protuberance ($< 3\text{mm}$) over the ovarian surface. The

suckling process, level of nutrition, body condition score (BCS), milk yield and season can deeply influence the resumption of ovarian activity and estrus during the postpartum period. In suckled buffaloes, the weaning of calf at 17-32 days postpartum induces early ovulation *i.e.*, 42 ± 8 days, compared to 55 ± 10 days in suckled buffaloes (Jainudeen *et al.*, 1993).

In acyclic suckled buffaloes, the temporary calf removal during 72 hours, at 91-93 days postpartum, induced ovarian cyclicity about 14 days earlier (Wongsrikeao *et al.*, 1990).

Wongsrikeao *et al.*, (1990) concluded that body condition is also an important factor that can influence the postpartum resumption of ovarian activity. In fact, in animals with a mean BCS of 3.3 (0 = extremely thin, 5 = fat), ovarian activity is resumed within 90 days, while animals with a BCS of 2.8 continued to be acyclic.

In the region where buffaloes were fed with a constant balanced diet, allowed to wallow and provided with shade, under summer conditions, females returned to estrus in a short postpartum interval with ovulation and showed a high calving rate (> 90%).

Table 2.2: Reproductive Parameters in Buffalo

Characters	River buffalo
First estrous (months)	15-18
Estrous cycle length (days)	21-37
Estrous duration (hours)	5-36
Ovulation (hrs after estrus)	11-24
Fertilization rate (%)	50-75
Blastocyst formation (days after fertilization)	6
Conception rate (%)	16-93

Implantation (days after fertilization)	25
Gestation (days)	315
First calving (years)	2.7-3.7
Calving interval (days)	550
Post-partum ovulation (days)	51.6 ± 2

2.2 Signs of estrus detection for AI

2.2.1 Estrus and estrus signs

Buffaloes are polyestrous but with a marked seasonal pattern of breeding activity, showing a distinct anestrus, and consequently, varied in display of estrus and calving rate. In SouthEast Asia, the breeding frequency of buffaloes is highest during the period from December to April (winter-spring) and lowest in the period from May to July (summer). The calving period usually last from December to January, the animals return to estrus and mating during spring. For the majority of remaining non pregnant buffaloes, they become anestrus due to the hot summer months (temperature extremes: 40°–46°C). Unobserved silent estrus and short duration of estrus are common in buffaloes during this period (El-Wishy, 2007).

The estrus cycle in buffaloes can vary from 16 to 28 days (Neglia *et al.*, 2007) and in buffaloes showing regular estrus cycles, the duration of estrus is typically 10–20 h if animals are cycling during the breeding season (Vale *et al.*, 1990). In females cycling during the non-breeding season the duration of estrus is highly variable, from 2 to 72 hrs (Baruselli, 2001a).

Therefore, other techniques have been developed, such as the use of teaser bulls (Zicarelli *et al.*, 1997), the radiotelemetry (Baruselli, 2001) and the use of pedometers (Di Palo *et al.*, 2001; Porto-Filho *et al.*, 2014).

The use of pedometers is largely applied in dairy cattle (Lehrer *et al.*, 1992). These instruments are localized at the tether of the animals and are able to record the motory activity of the subjects throughout the day. The rationale of their use is that the animals in estrous status show a higher motory activity than in other stages of the cycle.

In buffaloes, the pedometers are able to reveal the estrus status in 80% of the animals, with an accuracy of 75%. Furthermore, if a vasectomized bull is present in the herd, the sensibility of the system exceeds 90% (Di Palo *et al.*, 2001). The estrous cycle length is 21 days with heat duration of 12-24 hrs. The ideal buffalo produces a calf every 13 to 14 months. The factor which most strongly influences age at puberty is nutrition level. Buffalo exhibit seasonality in reproductive activity (Bhikane and Kawitkar, 2000).

The river type buffalo heifers are known to exhibit first estrus at 15 to 18 months, however, the first conception occurs at around 24 to 36 months of age (Campanile *et al.*, 2010). The delay in puberty and the consequent delay in conception is one of the problems that lead to low reproductive efficiency in the buffalo species (Barile, 2005).

2.2.2 Estrus behaviour

Not much research has been done for understanding buffalo behavior *vis-a-vis* AI for improving its success. However, recently, some isolated reports on use of automated systems for detecting estrus have come up on buffaloes.

In buffaloes, only 3.44% of the females present homosexual behavior than in cattle (Baruselli, 1994). The lower intensity of estrus behavior, together with the wide variation in the duration of the estrus in buffalo (6 to 48 hours), make estrus detection more difficult and impair the use of AI in this species.

Pedometry and pressure sensing radio telemetric heat watch system are examples of such systems being proposed. Pedometers in buffalo estrus detection have been applied in Italy (Di Palo *et al.*, 2001) and by radio telemetry in Brazil (Porto-filho *et al.*, 2014). These technologies are also practiced in some privately-owned buffalo farms in India.

Table 2.3: Percentage (%) of occurrence of heat signs in buffalos as observed by different authors

Heat signs	Jacomini (1989)	Baruselli (1992)	Vale (1994)	Crudeli (1996)	Crudeli (2003)
Stand to be mounted	100	100	100	41.6	86.1
Vulvar edema	87.5	79.3	67.0	66.6	80.7
Clear mucus discharge	12.9	13.8	76.1	16.6	6.6
Increased uterine tone	83.9	93.1	-	71.4	95.0
Vulvar hyperaemia	96.4	79.3	65.9	85.7	88.9
Mucus discharge at rectal palpation	96.8	69.0	-	66.6	40

The major signs of estrus are vulvar engorgement, frequent urination, bellowing, mounting, restlessness, mucus discharge and chin resting. (Madan and Prakash, 2007)

In a study, signs like bellowing/mooing, restlessness, clear mucus discharge, mounting, vulvar engorgement are the most reliable signs responsible for 85% of the total observations and frequent urination is most frequently observed sign during summer months. (Madan and Prakash, 2007)

Singh *et al.* (2000a) reported that signs of estrus in buffalo are less overt than in the cattle. Homosexual behavior between females is rarely seen. (Roy and Prakash, 2009; Noakes *et al.*, 2001).

Agarwal (1983) reported restlessness, bellowing, tail raising, vulvar swelling, decreased feed intake and frequent voiding of urine as the main behavioral signs of estrus detection in buffaloes.

The willingness of the female to stand for mating is regarded as a true sign of estrus (Perera, 2011). During summer, estrus is exhibited only during the night or in the early morning hours.

Silent heat is common during summer months (Zicarelli, 1997). The estrus signs are accompanied by changes in external genitalia *viz.* swelling of the vulva and reddening of the vestibular mucosa and changes in internal genitalia such as good uterine tone and coiling of the uterine horns. Due to vulvar swelling, the horizontal wrinkles which are present on its external surface disappear in estrus animal (Adams, 2000).

Secretion of mucus from the cervix during estrus is less copious than in cattle and does not usually hang as strands from the vulva, although a proportion of buffaloes may show mucus strands but can be seen by trans-rectal back racking of genitalia or when the buffalo sits (Perera, 2011). A few lactating buffaloes also exhibit *Doka* (temporary engorgement of teats without let down stimulus) 2-3 days prior to impending heat. However, individual variations are observed in the occurrence and the intensity of estrus signs in buffaloes.

Estrus behavior is less evident in the postpartum buffaloes. About 25-30% of estrus periods, which were associated with the first postpartum ovulation, were not detected, even when aided by a vasectomized bull (Jainudeen *et al.*, 1982). It was reported that the maximum diameters of the first, second, third and fourth ovulatory follicles were 13.5 ± 2.09 , 14.2 ± 1.58 , 14.8 ± 2.38 and 14.0 mm, respectively. This partially explains why relatively few postpartum buffaloes displayed estrus signs at

the time of the first ovulation (40 days postpartum), while all animals (100%) show estrus behaviour around the second, third, and fourth ovulation (Yindee *et al.*, 2011).

2.2.3 Service period

The interval between calving to conception is known as service period, whereas the interval between subsequent calvings is known as calving interval. Both these traits of economic importance appear to be prolonged in buffaloes compared to cattle and are widely variable across the different breeds (Bhat, 2010). The service period in Murrah buffaloes at well-organized farms is much lower compared to that observed for other breeds (Bhat, 2010). Average service period (days) 161.5 ± 14.0 is recorded in Jafrabadi buffaloes (Sethi, 2003; FAO, 2003).

The average service period in Murrah buffaloes is 115 to 230 days with an overall average of 132 days (Bhat, 2010), the average first service period is 201 days in Nili-Ravi, 193 days in Bhadawari and 198 days in Egyptian buffaloes (Sharma *et al.*, 1998).

2.3 Artificial Insemination

Artificial Insemination (AI) is the introduction and deposition of semen into the female reproductive tract without contact between the male and female.

Some diseases can be transmitted via semen and a hygienic and safe semen handling including control of the semen for contagious diseases is important. The fresh semen is also evaluated in terms of motility and quality. The spermatozoa in the collected semen are sensitive and must be handled with care. After collection the semen is cooled, frozen, and stored in liquid nitrogen (LN₂) in -196°C until it is time for thawing and insemination. It is important to avoid sudden temperature changes and cooling and thawing of the semen shall be made according to certain recommended approved regimes. Post-thaw motility should be at least 40%. It is

important to regularly check levels of LN₂ in storage containers (Galloway and Perera, 2003).

Artificial insemination is an important technique that offers several advantages over natural mating in developing countries for breeding dairy cows. A major benefit of the technique is that it offers excellent possibilities to improve the livestock genetically especially for the small-scale farmers so that their production and productivity are enhanced (Rodriguez-Martinez, 2012)

Similar surveys would be valuable in other developing countries to improve the quality and efficiency of AI services.

Anzar *et al.* (2003) reported that, in Pakistan, field AI conception rate is 29% and is affected by many factors including those related to farm, animal, semen, and AI technique.

The timing of AI relative to first detection of heat is known to be critical for achieving high conception rates (Peters, 2005).

Peters (2005) achieved higher conception rates in animals inseminated between 12- 18 hrs after detection of heat. The AIs were performed at time intervals ranging from 1 to 24 hours and, when grouped into 3 time intervals (0-6, 6-12, and 12-24), the respective conception rates were 60%, 49% and 51%. This pattern of conception in relation to timing of AI could have been most likely due to the herdsmen's inability to differentiate pro-estrus and estrus, and to make a correct judgement of the time of onset of estrus.

7 ± 5 hours duration of standing heat	Sperm transport and capacitation 6-18 hrs	Fertile life of ovum < 12 hrs
0 7 13	23 28	34 36

Fertile life of frozen- thawed sperm in female reproductive tract (Max. 36 hours)

Peters (2005) reported that the timing of insemination relative to first detection of heat is of a critical nature for achieving high conception rate. He studied a trend in conception rate was shown with time from detection of estrus to service; the conception rate increased as the interval increased from 6 up to 24 hours, and then declined.

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).

AI done 18-24 hours after detection by the farmer resulted in decreased conception rate. This resulted from the ignorance of farmers regarding the importance of correct timing of service and the poor communication between small holdings which are scattered and the AI service centres which are few in number. Attention therefore needs to be focused on addressing these deficiencies. Variations in fertility due to bulls were also observed. Of the seven bulls from which semen had been used for at least 20 inseminations, one had very poor conception rate (18.2%), while two others had conception rates below 45%. The continued use of such bulls in an AI programme is clearly unwarranted.

A regular programme should therefore be instituted to monitor each bull used in AI and to cull those that have low fertility. Semen produced locally gave higher conception rate than imported semen. Assuming that the imported semen was of good

quality at the point of origin, problems during subsequent transport, storage and/or handling could be responsible for the decline in fertility.

Although the number of observations was low, a tendency for higher conception rate with chilled than with frozen semen was seen. This further stresses the need for special care in all operations associated with frozen semen. The findings emphasized the need for provision of optimal conditions for transport and storage of semen, and also for routine monitoring of quality at the point of receipt and during storage.

The influence of the technician on the outcome of AI is well documented. Not only their skill, but also motivation, attitudes and the facilities available have profound influence on the outcome of AI.

However, the wide range of conception rates seen between individual technicians (27.8-58.5 per cent) is noteworthy (Alexander *et al.*, 1998).

Although further studies are needed to partition the effects of factors such as location, bull, semen type and technician in order to evaluate the true effects of technicians on conception rate, it is very conceivable that technicians may have contributed substantially to the variation in conception rates (Abeygunawardena, 2000).

2.3.1 Site of deposition in female reproductive tract

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).

It has traditionally been recommended to inseminate the female in the uterine body. There was a decline 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.

A study conducted by Souames *et al.* (2015) revealed that inseminators preferred depositing semen in the uterine body as opposed to the uterine horns (74 vs 15%). Other 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).

2.3.2 Single vs two inseminations

In a study reported by Adrian *et al.* (2017), out of total 50 number of AITs, 2% practiced single insemination, 16% practiced inseminating twice and 37% practiced inseminating thrice. This practice was adopted to improve the declining water buffalo population in Philippines.

2.3.3 Calving interval

The reports on calving interval in the buffalo present wide variations and calving intervals of as long as 839.5 days as were observed for Chinese swamp buffaloes (Qingkun *et al.*, 2002). It has been mentioned that Italian Mediterranean buffaloes with better nutritional management may have calving intervals as short as 400 days (Borghese *et al.*, 2011) as compared to calving intervals of 420-504 days in buffaloes elsewhere.

A large number of variables govern the calving intervals including season of calving, lactation yields and age at first calving (Shah, 2007). The calving interval in Murrah, Nili-Ravi and Egyptian buffaloes varies from 479 to 508 days (Bhat, 2010), and calving intervals of as long as 583 days were recorded for Surti buffaloes (Singh, 2002a).

Average dry period (days) 159.8 ± 10.9 and calving interval (days) 509.8 ± 20.1 are recorded in Jafrabadi breed of buffaloes (Sethi, 2003; FAO, 2003).

2.4 Skills and knowledge of AI technicians

The AI technicians must be well trained and have fresh knowledge in AI technique, hygiene routines, reproduction, heat detection, pregnancy checking, dairy animal nutrition and herd management. Correct AI work includes some minimum of equipment: a small portable LN₂ container, insemination gun, water thermos with hot water for thawing, thermometer, scissors, disposable gloves, disposable plastic sheets for the insemination gun, paper/paper towels, lubricant, recording files or record books, protective clothing, easily cleaned foot wear and soap (Galloway & Perera, 2003).

AI semen doses are sensitive to temperature changes and must be kept in adequate levels of LN₂ during storing to prevent damages on the spermatozoa (Galloway and Perera, 2003).

It is therefore essential that the AI technicians always have easy access to LN₂ so they can store the straws in LN₂ in a correct way. An organization responsible for the AI-work performed and supervision and control of the AI technicians in their work facilitates the goal to provide a good AI service. Hence, there is a guarantee of quality of the service of the AI technicians and the farmers know what to expect of the

AI service. Many factors can affect the conception results when using AI for breeding. As mentioned earlier, right insemination time is of great significance.

Other factors that affect the outcome are sperm quality and number of sperms in the insemination dose and handling of semen (Nadir and Degelos, 1993). The preferable place to deposit the semen is in the body of uterus (Zaini and Zahari 2007).

Some propounds deeper intra cornual insemination as a better place for semen deposition, but according to Hunter (2003) the insemination technique potentially could cause damage to the endometrium and in the worst cases cause perforation of the uterine wall.

Furthermore, palpation and manipulation of the ovaries, to determine where ovulation is expected to occur in the deep insemination, increase the risk of premature ovulation and a poor fertility result.

Farmers must be skilled in heat detection and keep proper records of fertility and reproduction in the herd. Farmers should look for heat in their herd for at least three times per day, in times other than during milking and feeding (Galloway and Perera, 2003).

Standing to be mounted is the primary sign of estrus, other signs of heat can be: swelling of vulva, mucus discharge, mounts other animals and frequent vocalization (Althouse, 2007).

2.4.1 Knowledge level regarding scientific AI process

Temkar (2000) observed that slightly less than two fifth (39.16 per cent) of the respondents had high level of knowledge regarding AI followed by 35 per cent and 25.84 per cent of them had low and medium levels of knowledge regarding AI, respectively.

Ganeshan and Seethalakshami (2002) revealed that slightly more than two-fifth *i.e.* 43.33 per cent of the respondents had high level of knowledge followed by 30.83 per cent and 25.84 per cent of them had medium and low levels of knowledge, respectively.

Khokhar (2007) observed that slightly less than two-third *i.e.* 63.34 per cent of the respondents were found to have medium level of knowledge level followed by an equal number *i.e.* 18.33 per cent of them had possessed high and low levels of knowledge, respectively.

Fertility is dependant on the inseminator also. 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.

2.5 Education and performance of skills

Temkar (2000) observed that level of education of the AI technicians was significantly correlated with their knowledge regarding AI.

Patel (2006) reported that there was a positive and significant correlation between the education and performance of the AI technician.

Khokhar (2007) found that education of dairy farmer had positive and significant relationship with their extent of adoption of dairy innovations.

Nehethe (2010) concluded that there was a positive and non-significant correlation between education of the dairy farmers and their management efficiency.

2.5.1 Experience in animal husbandry occupation and performance of skills

Khokhar (2007) observed that the relationship between experience of the AI technician in dairy occupation and their extent of adoption of dairy innovations was found to be positively significant.

Nehethe (2010) stated that there was a positive and highly significant correlation between experience in dairy farming of the dairy farmers and their management efficiency.

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 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).

2.5.2 Experience as an AI technician and performance of skills

The technique of inseminating is a skill requiring adequate knowledge and experience. Semen must be deposited within the tract of the female animal at the suitable location and at the proper time to obtain acceptable conception rates. Improper AI techniques can negate all other efforts to obtain conception.

Halyal (1968) observed that the experience on the present post of AI technicians was found to have a non-significant influence on the rate of adoption of some improved techniques.

Durgga (2009) found positive and highly significant relationship between experience of the AI technicians in dairy farming and their extent of adoption of crisis management.

2.5.3 Training(s) received and performance of skills

Supervised instruction and guidance are essential because without adequate training, valuable AI equipment and semen could be seriously damaged. In order to ensure high fertility, frozen semen requires very special storage and handling techniques. Adequate training is also essential to minimize risk of injury to either a valuable animal or to AI technician.

AI training schools are available from several semen suppliers. The objectives of these schools are to teach the skills required to handle semen, inseminate animals and manage a successful AI program (Souames *et al.*, 2015).

Insemination technique, developing through live animal practice, the ability to skillfully and accurately place semen at the proper location within the reproductive tract using sanitary and correct techniques.

Semen handling, developing through practice, the ability to properly handle, thaw and prepare semen for insemination, according to the recommendations of semen-producing organizations.

Reproductive management training in the importance of heat detection, herd health, and total herd management for the development and continued success of an AI program.

Sutthianlal (2010) reported that 40.38% of the AI technicians had received training more than once followed by 38.47% and 21.25% with no training and one training, respectively.

2.6 Seminal characteristics and cryopreservation of semen straws

Buffalo semen is milky white, never yellow. Its consistency depends on its content of spermatozoa and is affected, among other factors by frequency of

ejaculation. The average sperm concentration is about 800 million/ml. Values as high as 1500-2000 million sperm/ml and as low as 200 million sperms/ml have been recorded. First ejaculates contain higher number of spermatozoa per ml compared to second ones.

The quality of frozen semen when it arrives at your farm or ranch is determined by the bull and organization that processed it. But once it arrives, it is up to you to take proper steps to ensure its viability. Frozen bull semen can be stored indefinitely, if it is maintained constantly at very low temperatures. The critical temperature is approximately -196 °C. Semen which is exposed to temperatures warmer than -196° C (even for a short time) and then returned to the storage tank may be damaged. The extent of damage depends upon how long the semen is exposed to the elevated temperatures. Although it is easy to maintain frozen semen at a safe temperature, it is also easy to destroy it in a few moments of carelessness.

Frozen bovine semen has been widely used in AI. But freezing-thawing processes lead to the generation of reactive oxygen species (ROS) that impair sperm motility, membrane integrity, and fertilizing potential (Upreti *et al.*, 1998; Chatterjee *et al.*, 2001).

Bucak *et al.* (2007) reported that antioxidant treatment with trehalose significantly elevated vitamin E concentrations of sperm and improved post-thawed ram sperm motility.

Liposome-based semen extender was found to be a suitable alternative to egg yolk-based extender for semen cryopreservation in buffaloes (Kumar *et al.*, 2015).

Rhodes *et al.* (1985) found significantly poorer results in respect of freezability of ejaculates and post-thaw quality of *Bos indicus* semen than those of

Bos taurus. As compared to ox bull semen, buffalo semen has been reviewed to have low freezability and even fertility (Sengupta and Sukhija, 1988).

According to several reports, sperm concentration is higher in summer (Soysal *et al.*, 2005 and da Luz PA *et al.*, 2012) or spring (Rajamahendran, 1987).

Initial motility and live sperm percent are also optimal during winter (da Luz PA *et al.*, 2012). Improvement of semen quality during winter and /or spring is consistent with higher conception rates of buffaloes observed during these seasons (Narasimha Rao *et al.*, 1979).

The percentage of abnormal spermatozoa in buffalo semen varies between 3 and 26% (Bongso *et al.*, 1994) being less frequent in winter (Malfatti *et al.*, 2006). Compared to Egyptian buffalo bulls, the quality of the Indian Murrah semen is optimal in spring (Rajamahendran, 1987).

Buffalo bulls in regular use as semen donors at AI centers can be ejaculated thrice in rapid succession, two times a week without serious effect on their semen quality (Yassen, 1997).

2.7 Semen Evaluation

2.7.1 Computer assisted semen analysis (CASA)

Computer assisted semen analysis is the potential tool for accurate semen analysis (Amann and Waberski, 2014).

There are well-defined cluster analysis techniques for using two or more of the motility descriptors and defining sperm subpopulation structures (Abaigar *et al.* 1999; Roca *et al.* 2006).

CASA consists of optics, light source and the computer hardware and motile, morphology and vitality that can analyses module that contain software is a

combination. This method was first developed using multiple time-exposure photomicrography to follow spermatozoid movements.

The images obtained allow the analysis of several parameters, including semen concentration, semen motion and some morphology, particularly sperm head morphology because of the post-acquisition processing of digitalized data, the CASA is able to objectively determine morphological parameters or distinguish subpopulations in sperm head motion, which are not measurable or observable manually.

CASA produces similar and rapid results when repeated, precisely evaluates the sperm attributes and having little error due to human judgment. CASA systems have evolved over approximately 40 years, through advances in devices to capture the image from a microscope, huge increases in computational power concurrent with amazing reduction in size of computers, new computer languages, and updated/expanded software algorithms. Remarkably, basic concepts for identifying sperm and their motion patterns are little changed. Older and slower systems remain in use.

Majority of the spermatology laboratories and semen processing facilities have a CASA system, but the extent of reliance thereon ranges widely. Each marketed system is different. Modern CASA systems can automatically view multiple fields in a shallow specimen chamber to capture strobe-like images of 500 to >2000 sperm, at 50 or 60 frames per second, in clear or complex extenders, and in less than 2 minutes, store information for ≥ 30 frames and provide summary data for each spermatozoon and the population. A few systems evaluate sperm morphology concurrent with motion. CASA cannot accurately predict 'fertility' that will be obtained with a semen sample or subject. However, when carefully validated, current CASA systems provide

information important for quality assurance of semen planned for marketing, and for the understanding of the diversity of sperm responses to changes in the microenvironment in research. The four take-home messages from the review are: (1) animal species, extender or medium, specimen chamber, intensity of illumination, imaging hardware and software, instrument settings, technician, *etc.*, all affect accuracy and precision of output values; (2) semen production facilities probably do not need a substantially different CASA system whereas biology laboratories would benefit from systems capable of imaging and tracking sperm in deep chambers for a flexible period of time; (3) software should enable grouping of individual sperm based on one or more attributes so outputs reflect subpopulations or clusters of similar sperm with unique properties; means or medians for the total population are insufficient; appropriate hardware to capture images and process data apparently are available (Amann and Waberski, 2014).

The disadvantages of CASA are related to the cost of the equipment, the extreme need of validation, quality control and standardization of the measures realized. It is vital to use appropriate optics and the best illumination to enhance the contrast of the spermatozoa heads, which in turn facilitates the manual selection of thresholds. However, the choice of frame rate is still conflicting, as it is not only equipment-dependent but related to species and experimental condition as well reported by Kraemer *et al.* (1998).

In general, more variations are observed for the analysis of different fields than for the repeated analysis of the same field, so the larger the number of cells analyzed, the more reduced the coefficient of variation. Consequently, the precision of the results increases as the number of fields and cell analyses increase. In bull, the

measure of 30 fields and approximately 300 cells are recommended (Verstegen *et al.*, 2002).

Shukla and Mishra (2005) reported overall total sperm abnormalities as 12.57 ± 0.25 per cent in Murrah buffalo bull semen. The average values of head, mid piece and tail abnormalities were 1.27 ± 0.09 , 2.06 ± 0.14 and 9.22 ± 0.22 per cent, respectively.

Patel *et al.* (2012) studied semen ejaculates (90) of 5 mature bulls each of Jafarabadi and Mehsana breeds collected at weekly interval during autumn season. The mean ejaculate volume (ml), mass activity (score 0-5), individual sperm motility (%), sperm concentration (million/ml), live sperm (%) and abnormal sperm (%) in Jafarabadi buffalo bulls' semen were 4.81 ± 0.21 , 3.30 ± 0.08 , 74.33 ± 0.75 , 1466.80 ± 72.43 , 84.93 ± 0.59 and 10.67 ± 0.43 respectively. The corresponding values for Mehsana buffalo bulls' semen were 5.01 ± 0.23 , 3.23 ± 0.12 , 73.83 ± 0.67 , 1307.43 ± 94.27 , 82.47 ± 0.67 , 10.83 ± 0.38 and 71.53 ± 0.89 .

The ejaculate volume had significant negative correlations ($P < 0.05$) with most traits in both the breeds; while mass activity showed significant positive correlations with initial motility, sperm concentration and live sperm percentage. The motility and sperm concentration were positively ($P < 0.01$) associated, while live sperm and abnormal sperm were negatively ($P < 0.05$) correlated.

Mahmoud *et al.* (2013) conducted a study to assess the relationship between frozen thawed sperm characteristics and *in vivo* fertility through AI. Semen samples from four buffalo bulls were extended with Soybean based Bioxcell extender (IMV, France) to the desired sperm concentration per milliliter and subsequently cryopreserved. They recorded individual motility, live sperm and HOST reactive sperm before and after freezing as 65.8 ± 0.9 , 70.9 ± 0.7 , 84.54 ± 1.09 and $42.51 \pm$

0.88, 61.76 ± 1.22 , 52.70 ± 0.86 per cent, respectively. Post-thaw sperm abnormalities were 15.19 ± 0.64 per cent and overall pregnancy rate was 44.5 per cent.

Mahamoud *et al.* (2013) noticed highly significant ($P < 0.001$) difference in membrane integrity before and after freezing. Significant correlations were found between motility and sperm abnormalities ($r = -0.64$; $P < 0.05$) and membrane integrity ($r = 0.64$; $P < 0.05$). A significant negative correlation ($r = -0.73$; $P < 0.01$) was found between sperm abnormalities and membrane integrity, and a positive correlation between pregnancy rates and live sperm percentages ($r = 0.65$; $P < 0.05$). Thus, motility and live sperm percentage can be used as predictive measures in semen evaluation.

To reduce variability and misinterpretation of results, a larger number of cells have to be counted. Staining methods are certainly species-dependent. A number of stains have been suggested for sperm morphology assessment; however, few researches indicate these stains do not necessarily provide the appropriate gray-level contrast for accurate computer-assisted morphometric analysis. Papanicolaou's staining and haematoxylin are most used for morphologic assessment in CASA by Davis and Katz (1993b). Magnification also affects operation. Bull sperm heads must be analyzed at 60 X. The advantage of lower magnification is the decrease in time needed to analyze the sample. Correct illumination and focus are essential to a consistent reading.

Microscopic evaluation of sperm motility is an unreliable parameter for fertility prediction due to lots of error and bias (Coetzee *et al.*, 1999).

However, it is not clear that which characteristics of sperm motion assessed by CASA are of value in predicting fertility. Live sperm count and hypo-osmotic swelling tests evaluate viability and functional integrity of sperm plasma membrane, respectively. However, their relationship with fertility rates is not established. The

present study is conducted to assess the relationship between sperm motion traits and viability with fertility rates.

Muino *et al.* (2008) studied various sperm motion traits (mean \pm SE) assessed through CASA and concluded that the average fertility rate (%), individual motility (%), progressive motility (%), VAP ($\mu\text{m/s}$), VSL ($\mu\text{m/s}$), VCL ($\mu\text{m/s}$), ALH (μm), BCF (Hz), STR, LIN, sperm size (μ), live (%) and HOS reactive sperm (%) were as 44.10 ± 4.75 , 45.20 ± 3.50 , 23.55 ± 2.16 , 94.48 ± 2.87 , 79.07 ± 2.49 , 160.06 ± 5.99 , 6.56 ± 0.26 , 35.06 ± 0.43 , 83.32 ± 0.68 , 52.68 ± 0.96 , 6.00 ± 0.07 , 67.31 ± 2.25 and 57.47 ± 2.64 , respectively.

Motility indicates energy status of sperm and higher proportion of progressively motile sperm increases the chances of fertilization (Muino *et al.*, 2008). Post thaw viability of sperm in bulls has been reported as 32 to 38 per cent (Kumar *et al.*, 2004).

The membrane integrity in buffalo bull sperm have been observed as 31.22 to 47.63 per cent (Kumar *et al.*, 2003) subjected to 100 mOsmol/L solutions.

The average fertility rate was 44.10%. The pregnancy rates in buffaloes have been reported from 18-56 per cent (Berber *et al.*, 2002; Karena and Darwish, 2010).

The significant positive correlation between HOS reactive sperm and individual ($r = 0.705$) and progressive motility ($r = 0.567$) proves the fact that spermatozoa with biochemically active plasma membrane will have better progressive motion. A significantly higher negative correlation existed between size and STR which indicate that smaller sperm have more tendencies to follow a linear path (Karena and Darwish, 2010).

Barrat *et al.* (1993) found the total number of spermatozoa and VAP to be predictors of chance of pregnancy, whereas Irvine *et al.* (1994) found mean head area

and the manually determined percentage of progressively motile spermatozoa as being significant covariates.

Other parameters as ALH, VSL, VCL and linearity (LIN) have also been reported to be correlated with fertility (Krause, 1995). Study indicated that fertility rate had no significant correlation with sperm motion traits and viability.

Thawing at 30-37 °C for 10-60 seconds is commonly recommended for bovine semen. Temperature control of thawed semen is also having great importance. This should not be allowed to cool below the final temperature achieved during thawing; otherwise substantial sperm losses can occur. Cold shock can easily be induced if post thaw cooling occurs (Nebel, 2007).

In addition, controlling the temperatures of semen during withdrawal from LN₂ container is important. Temperature at the top third of the neck of the LN₂ container are high enough for ice recrystallization to occur in the straws which results in markedly reduced post-thaw survival (Nebel, 2007).

2.7.2 Initial and post-thaw motility of bovine semen

The characteristics of frozen–thawed semen of fertile (conception rate of >55 per cent) and sub fertile (conception rate of 28 per cent to 35 per cent) buffalo bulls *viz.* motility parameters; head biometry, acrosome, plasma membrane, and DNA integrity were compared.

The percentage of intactness of sperm acrosome was higher in case of fertile bulls, whereas apoptotic sperm were higher in sub fertile bulls. The sperm DNA integrity was not different between fertile and sub fertile bulls. This study showed that the total motility, average path velocity, curvilinear velocity, straight linear velocity, length and width of sperm head, acrosome integrity and percentage of apoptotic

sperm are useful for evaluating bulls' semen quality to reduce the risk of using semen of poor-fertility bulls in AI programme (Kumar *et al.*, 2014).

Artificial Inseminations in over 100 buffaloes by reducing the number of sperms per straw from 25 million to 15 million had no effect on conception rate (Sharma, 2008). Currently, 20 million sperms are being used as standard AI dose. This can have great implications for buffalo male germplasm dissemination, as the number of available doses can be increased significantly by reducing the number of sperms per straw.

An ability of spermatozoa to undergo freezing at ultra-low temperature varies from bull to bull. Even the age (Inskaya and Osipova, 1972), season (Bochnke *et al.*, 1976), breeds (Patel *et al.*, 2000) and species (Shelke and Dhimi, 2001a) are also some of the established factors, which affect the freezability of bovine semen.

Dhimi *et al.* (1990) reported post-thaw motility of 52.28 ± 1.57 , 58.93 ± 1.98 ($P < 0.01$) in Surti bulls. Post-thaw motility had significant ($P < 0.01$) positive correlation with initial motility ($r = 0.570$), Live sperm per cent ($r = 0.540$) and tail abnormalities ($r = 0.240$) as well as negative correlation with mid-piece abnormalities ($r = -0.250$) and total sperm abnormalities ($r = -0.340$).

Mohan *et al.* (1994) reported the post-thaw motility of 38.39 ± 1.42 , ($P < 0.05$) in Murrah bull semen.

Dhimi *et al.* (1998) recorded initial motility and post-thaw motility of 83.80 ± 0.72 and 47.26 ± 1.59 per cent in Murrah bulls' semen, respectively, the differences being significant at both the levels. Singh *et al.* (2000b) reported that the mean value of the post-thaw sperm motility 43.92 ± 1.75 per cent in buffalo bull semen extended in Tris. Maurya *et al.* (2003) reported progressive sperm motility in fresh and post-

thaw semen of Murrah buffalo bull as 65.32 ± 3.01 and 33.80 ± 2.32 per cent and live sperm 70.23 ± 2.79 and 49.91 ± 2.44 per cent, respectively.

Murugan and Raman (2003) reported initial and post-thaw motility in adult Murrah buffalo semen as 68.70 and 50.60 per cent, respectively.

Khan and Ijaz (2007) reported the mean percentages of progressively motile spermatozoa at initial and post-thaw stage in semen of Nili-Ravi buffalo bulls in Tris diluent as 81.00 ± 1.57 and 60.10 ± 1.34 , respectively.

Meena *et al.* (2010) reported mean values and post-thaw motility values as 78.39 and 55.45 per cent in Murrah buffalo bull semen extended in Tris.

The antioxidant treatment resulted in greater sperm motility, acrosomal membrane integrity and plasma membrane integrity with an improvement of 9.73, 11.38, and 8.01% over the control group ($P < 0.05$), respectively.

No difference was observed for sperm motility between trehalose 50 and 100 mM groups. For 25 and 200 mM trehalose groups, sperm acrosome integrity and motility did not differ from the control. For 100 mM trehalose groups, sperm membrane integrity exhibited improvement, compared with control and other treatment groups ($P < 0.05$). However, no difference was observed for the percentages of intact-membrane sperm among the control and other groups.

Dhami *et al.* (1990) reported post-thaw motility of 52.28 ± 1.57 , 58.93 ± 1.98 and 61.36 ± 2.14 per cent ($P < 0.01$) in Surti, Jersey and crossbred bulls. Vyas *et al.* (1992) reported initial motility of 71.03 ± 1.37 per cent and post-thaw motility 34.89 ± 1.42 per cent in triple crossbred ($1/2$ HF x $1/4$ J X $1/4$ H) bull semen.

Mohan *et al.* (1994) reported the post-thaw motility of 38.39 ± 1.42 , 44.72 ± 1.48 and 35.50 ± 2.52 per cent ($P < 0.05$) in Murrah, Friesian and crossbred bulls semen, respectively.

Dhami *et al.* (1995) studied freezability of semen of Friesian bulls with and without additives. The initial and post-thaw sperm motility was 81.11 ± 0.86 and 51.11 ± 1.64 per cent, respectively. Mean conception rate was 58.82 per cent. Singh *et al.* (1997) reported the mean individual motility, live sperm and abnormal sperm percentage as 79.80 ± 0.01 , 84.40 ± 0.01 and 2.03 ± 0.01 in neat semen of Sahiwal x Jersey halfbred bulls, which dropped significantly ($P < 0.01$) following freezing to 55.23 ± 0.01 , 52.90 ± 0.01 and 3.90 ± 0.01 %, respectively.

Veeraiah *et al.* (1999) reported the value of prefreeze and post-thaw motility in Tris with 6 per cent glycerol and 20 per cent yolk as 70.69 ± 0.96 and 42.08 ± 0.89 per cent, respectively, in Ongole bull semen.

Rana *et al.* (2003) reported initial, prefreeze and post-thaw motility in Gir bull semen as 71.50 ± 0.90 , 61.00 ± 1.06 and 46.00 ± 1.18 per cent, respectively.

There is large variation in post-thaw motility and rate of decline in motility after freezing of semen between different breeds/reports. Murugan and Raman (2003) reported initial, prefreeze and post-thaw motility in adult Murrah buffalo semen as 68.70, 68.00 and 50.60 per cent, respectively.

Table 2.4: Reported mean motility profile (%) of different breeds of buffalo bull semen

Buffalo breed	Initial motility (%)	Post-thaw motility (%)	Author(s) and year
Surti	76.90	44.50	Dhami <i>et al.</i> , 1988
Mehsana	77.60	54.20	Bhavsar <i>et al.</i> , 1989
Murrah	84.90	37.20	Bhosrekar <i>et al.</i> , 1990
Surti	74.70	35.00	Singhala, 1993
Murrah	75.90	34.50	Kumar <i>et al.</i> , 1996
Jafrabadi	65.80	36.90	Merja, 1997

Murrah	77.30	53.00	Rasul <i>et al.</i> , 2001
Jafrabadi	56.40	28.20	Shelke and Dhami 2001
Murrah	72.50	61.50	Pant <i>et al.</i> , 2002
Murrah	71.10	46.80	Rohilla <i>et al.</i> , 2005
Murrah	73.40	42.10	Koonjaenak <i>et al.</i> , 2006
Surti	77.40	59.20	Nandre, 2007
Egyptian	82.25	36.60	El-sisy <i>et al.</i> , 2010
Murrah	72.14	72.25	Swelum <i>et al.</i> , 2011

Raval (2006) reported the mean percentages of progressively motile spermatozoa in fresh and post-thawed semen of triple crossbred bulls in Tris diluent as 77.92 ± 0.73 and 46.39 ± 1.27 per cent, respectively.

Sugulle *et al.* (2006) reported progressive sperm motility in fresh, equilibrated and post-thawed semen of crossbred bull as 65.0 ± 7.00 , 58.0 ± 8.00 and 48.0 ± 8.00 per cent, respectively.

Nagendrakumar and Kathiresan (2009) reported overall initial and post-thaw motility of Jersey bull semen as 63.33 ± 0.53 and 60.00 ± 0.59 per cent, respectively; while Rao *et al.* (2011) reported the values of 77.27 ± 0.66 and 57.85 ± 1.61 per cent, respectively.

*MATERIALS
AND
METHODS*

The present research on “Study on Quality of Frozen Semen and Efficiency of AI Technicians and their Impact on Reproductive Performance of Buffaloes under AI Network Programme” was conducted over a period of four months from April to July, 2017. This chapter contains information regarding selection of area, techniques employed for collection of data, sampling techniques, computer assisted sperm analysis and appropriate tools for the analysis of data as under:

3.1 Location and climate

3.2 Collection of data and sampling procedure

3.3 Selection and measurement of attributes of AI technicians

3.4 Computer assisted analysis for frozen-thawed semen

3.5 Statistical tools and techniques

3.1 Location and climate

The research was carried out in field areas of Sabarkantha, Panchmahal and Arvalli districts of Gujarat and computer assisted sperm analysis (CASA) was done at laboratory situated in Sabarmati Ashram Gaushala Unit office, Ashram road, Ahmedabad during April to July 2017.

Sabarkantha is located 23.6 N latitude, 72.95 E longitude and 234 meters above sea level with overall maximum temperature 39.9 °C and minimum temperature 20.4 °C, humidity 45% and wind speed 126.9 kmpd. Arvalli is located 23.47 N latitude, 73.3 E longitude and 197 meters from sea level. Overall average temperature of the year 2017 is maximum 38.1 °C and minimum 21.2 °C with humidity 46% and wind speed 135.3 kmpd. Panchmahal is located 22.45 N latitude,

73.36 E longitude and 73 meters from sea level. The temperatures are highest on an average in May, at around 33.4 °C. January had the lowest average temperature (20.2 °C) of the year. Overall average temperature of the year 2017 is maximum 32.6 °C and minimum 19.7 °C with humidity 50.3% and wind speed 259.3 kmpd.

3.2 Collection of data and sampling procedure

Personal interview technique was used as a tool through which first-hand information on the attributes of AI technicians was collected. Twenty eight questions were asked to the AI technicians which included general data about the inseminator (age, education, trainings, experience pertaining to AI, professional duties, types of gynaeco-clinical activities performed by the AI technicians, heat expression signs observed by AI technicians, AI practices and percentage etc.). The semi-structured proforma was prepared by keeping in view the objectives of the study and was common for all the technicians. The questionnaire was prepared after obtaining guidance from the advisory committee and veterinarians in the field and also referring available literature pertaining to this topic (**Annexure-I**). Apart from interview schedule, observation technique was also used for data collection. Wherever required, data from secondary source *i.e.* Information Network on Animal Production and Health (INAPH) network of National Dairy Development Board (NDDB), Anand was also collected.

3.3 Selection and measurements of attributes of AI technicians

3.3.1 Measurement of independent variables

3.3.1.1 Age

The age was taken as the completed number of years of the AI technicians on the date of interview. The respondents were categorized into four groups (**Table 3.1**).

3.3.1.2 Education

The education of the AI technicians was measured as the level of education in terms of educational standard that technicians had passed. Based on education obtained, technicians were classified into four groups (**Table 3.1**).

3.3.1.3 Training

This refers to the types of trainings received by the AI technician, prior to and during his job as an AI technician regarding the scientific artificial insemination skills. AI technicians were classified into three groups (**Table 3.1**).

3.3.1.4 Experience

This refers to the number of years passed by the AI technician has carried out the AI in the field. The AI technicians were classified into five groups (**Table 3.1**).

Table 3.1 Selection of variables, its measurements and grouping

Sr. no.	Variables	Measurement of variable	Groups
3.5.1	Age Groups	21 to 30 years	I
		31 to 40 years	II
		41 to 50 years	III
		51 years and above	IV
3.5.2	Education	Less than 10 th Standard	I
		Secondary(10 th pass)	II
		Higher secondary (12 th pass)	III
		Graduate and above	IV
3.5.3	Training	Basic AI Training	I
		Refresher Training(s)	II
		Both	III
3.5.4	Experience	Up to 5 years	I
		6 to 10 years	II
		11 to 15 years	III
		16 to 20 years	IV
		21 years and above	V

To determine the knowledge level of the AI technicians regarding various components, the process was divided into following steps and then recorded in proforma (**Annexure-I**).

1. Basic information regarding AI
2. Personal care and hygiene
3. Care of AI instruments and Liquid Nitrogen (LN₂) containers
4. Refilling of LN₂ containers
5. History taking
6. Observations of estrus signs
7. Thawing temperature and time taken for thawing
8. Loading of AI gun
9. Insertion of gun and deposition of semen into female reproductive tract
10. Site of semen deposition
11. Number of inseminations in existing estrus
12. Examination of gun and sheath post AI
13. Disposal of sheath, gloves, straws and cleaning of AI gun

Depending upon individual's response, classification was made and means, standard deviation and standard error were calculated by statistical analysis.

3.4 Computer assisted analysis for frozen-thawed semen

3.4.1 Semen Sample

Frozen french mini straws were collected randomly from the selected AI technicians (31) working at the different AI centers of Sabarkantha, Arvalli and Panchmahal districts of Gujarat. Samples (62) were collected and stored in separate pencil goblets with proper identification tags in LN₂ container. The samples were brought to SAG, Ahmedabad for computer assisted semen analysis.

The IVOS II hardware and software have been designed to provide users with a system that offers high-end performance while maintaining its ease of use. The intuitive Windows-based software promotes fast learning and quickly increases the confidence level of users. Since the settings for the integrated optics are made through the software, mechanical adjustments are rarely required. With a minimum investment of time, even users unfamiliar with computers will be performing fast, accurate and reliable sperm analyses. You don't need to be a computer expert to analyze sperm with the IVOS II

- Smoother, faster integrated stage featuring acceleration and deceleration. It has a new stage drive motor, the IVOS stage now can move from maximum speed down to start speed in the blink of an eye.
- Enhanced processing power is supplied by the latest generation of Intel technology - the "i Series." With a minimum of 4 simultaneous processing threads, the IVOS is faster than ever.
- Machine has a rearrangement of user controls and the addition of inputs on the IVOS front panel make for a better user experience:
 - On / off switch added to the front panel
 - Stage LOAD button separated from the JOG buttons to avoid inadvertent stage loading/unloading.
 - Four high speed USB 2.0 ports for easy data transfer and connection to external devices
 - Blu-ray DVD burner/player - save up to 25 GB of data to Blu-ray Disc
 - Ruggedized Medical wide range AC/DC power supply
 - High definition, wide screen monitor
 - Wireless keyboard and mouse means less work area clutter

- 1 Terabyte 6.0 GB/sec 7200 RPM hard drive
- Dual Gigabit Ethernet ports
- Dual digital monitor DVI connections plus display port connection
- RAID drive option allows dual mirrored drive for reliability
- Windows 8

20 μ l of semen sample was taken by micro pipette and mixed with same amount of freshly prepared TRIS buffer. Thawing was carried out at 37°C for 30 seconds in water bath. Prepared sample was put on pre-warmed Leja slide (8 chamber) for Computer Assisted Semen Analysis and analysis was done one by one for all the samples.

Average path velocity (VAP) is the velocity over a calculated path. Curvilinear velocity (VCL) is the velocity over the total distance moved. It includes all deviations of sperm head movement. Straight line velocity (VSL) is the velocity calculated using the straight line distance between the beginning and the end of the sperm track.

Collected frozen-thawed semen straws were evaluated for total motility, progressive motility, viability, average path velocity (VAP), straight line velocity (VSL), curvilinear velocity (VCL), lateral amplitude (ALH), beat cross frequency (BCF), straightness (STR), linearity (LIN), head elongation, area. Both total motility and progressive motility were determined through computer assisted semen analysis (CASA; Hamilton-thorne IVOS II). Frozen-thawed semen (20 μ l) from each straw was mounted on a disposable CASA slide (Leja-8; IMV Technologies, France) to analyze the total motility and progressive motility. Five randomly selected fields were scanned per straw. Sperm abnormality was established by analyzing through CASA machine.

Head, mid piece and tail abnormalities and location of cytoplasmic droplets on spermatozoa were analyzed by CASA machine. Morphologic defects detected in the spermatozoa included both primary and secondary abnormalities. The mean of 25 scans for the total motility and progressive motility and the mean of two replicates (straws) for the per cent motility and per cent progressive motility, abnormality and concentration for each semen straw were used for the statistical analysis.

List of sperm characteristics measured by CASA are as under:

1. Bent tail concentration (million/ml)
2. Bent tail percent of total sample
3. Bent tail count (million)
4. Coiled tail concentration (million/ml)
5. Coiled tail percent of total sample
6. Coiled tail count (million)
7. Average path velocity (VAP) (m/s)
8. Straightline velocity (VSL) (m/s)
9. Curvilinear velocity (VCL) (m/s)
10. Amplitude of lateral head (ALH)
11. Beat cross frequency (BCF) (Hz)
12. Straightness (STR)
13. Linearity (LIN)
14. Elongation (ELG)
15. Area (ARE)
16. Post-thaw motility (%)
17. Motile concentration (million/ml)
18. Motile count
19. Motile mean elongation
20. Progressive concentration (million/ml)
21. Progressive count (million)
22. Progressive motility (%)
23. Slow concentration (million/ml)

24. Slow percent of total sample
25. Total concentration (million/ml)
26. Total count

3.5 Statistical tools and techniques

The data collected through interview schedule and direct observations was transferred on the master sheet to describe personal and field oriented characteristics of the AI technicians. Thereafter, frequency distribution table was counted and percentage was calculated. Collected data in the proforma and data generated after CASA were compiled, tabulated and analyzed using appropriate statistical tools and techniques like percentage, incidences, mean, standard deviation, standard error, one-way ANOVA and Karl Pearson's correlation coefficient in consultation with statistician in view of the objectives of the study. The statistical analysis was performed using IBM SPSS Statistics version 24.0 software package, 2016.

Annexure-1

PROFORMA FOR AI TECHNICIANS

Serial No:

Name:

District:

Tehsil:

Village:

-
1. Age: _____
 2. Education: (a) Less than 10th (b) 10th (c) 12th (d) Graduation
 3. Which type of training obtained?: (a) Basic (b) Refresher
 4. How many times refresher training obtained? _____
 5. Name of the institute where training(s) obtained:

 6. Duration of training(s): (a) < 30 days or 30 days (b) 45 days (c) 2 months
(d) 3 months (e) 6 months (f) 1 year
 7. Experience related to AI work (in years) :
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
More than 20
 8. Name the activity involved other than AI:

 9. What type of Gynaecological and Obstetrical work performed?
(a) Infertility (b) PD (c) Dystocia (d) Torsion (e) ROP (f) Prolapse
 10. Do you take history of animal before insemination? (a) Yes (b) No
 11. Detection of animal in heat: (a) By history (b) By external signs (c) Both
 12. How do you detect that the animal is in heat?

(a) By external signs i. Restlessness ii. Mounting on other animals iii. Clear vaginal discharge	(b) By rectal palpation i. Tonicity of uterus a) Yes b) No c) Occasional ii. Ovarian structure (Follicle/CL)
---	---

iv. Mooring/Ballowing	a) Yes b) No c) Occasional
v. Swollen vulvar lips	iii. Any other
vi. Reddening of vulvar mucosa	

13. Are the aseptic precautions taken before insemination?: (a) Yes (b) No

14. Cleanliness and sterilization of AI instruments:

Instrument	Good	Moderate	Poor
AI gun			
Forceps			
Plastic Sheath			

15. Site of insemination in female genitalia:

- (a) On external os (b) Mid cervix (c) Utero-cervical junction
(d) Uterine body

16. Timings of insemination post estrus :

- (a) Less than 6 h (b) 6 to 12 h (c) 12 to 18 h
(d) 18 to 24 h

17. Number of animals inseminated and pregnancy rate obtained during last 5 years:

Animal	2012	2013	2014	2015	2016
Buffalo					

18. How many times animals are inseminated in single estrus?

- (a) Once (b) Twice (c) More than 2 times

19. If inseminated twice, then duration of insemination between two AI:

- (a) 6 to 8 h (b) 9 to 12 h (c) More than 12 h

20. How many straws do you use in single insemination? (a) One (b) Two

21. Is the insemination(s) followed by natural service?: (a) Yes (b) No

22. % of AI

At AI center	Door step

23. Liquid nitrogen:

Refilling of LN ₂ container at AI center		Refilling of LN ₂ container carried in the field	
---	--	---	--

24. Temperature and time taken for thawing of semen straws:

- (a) 37 °C and 30 sec (b) 37 °C and 1 minute
(c) 36 °C and 1 minute (d) Any other: _____

25. Time spent in AI activities:

- (a) 6 to 8 h/day (b) 9 to 12 h/day (c) 13 to 15 h/day (d) More than 15 h/day

26. Calving rate (from data): _____

27. Problem faced : _____

28. Suggestion(s) if any, for improvement :

Equipments and other items used in Research

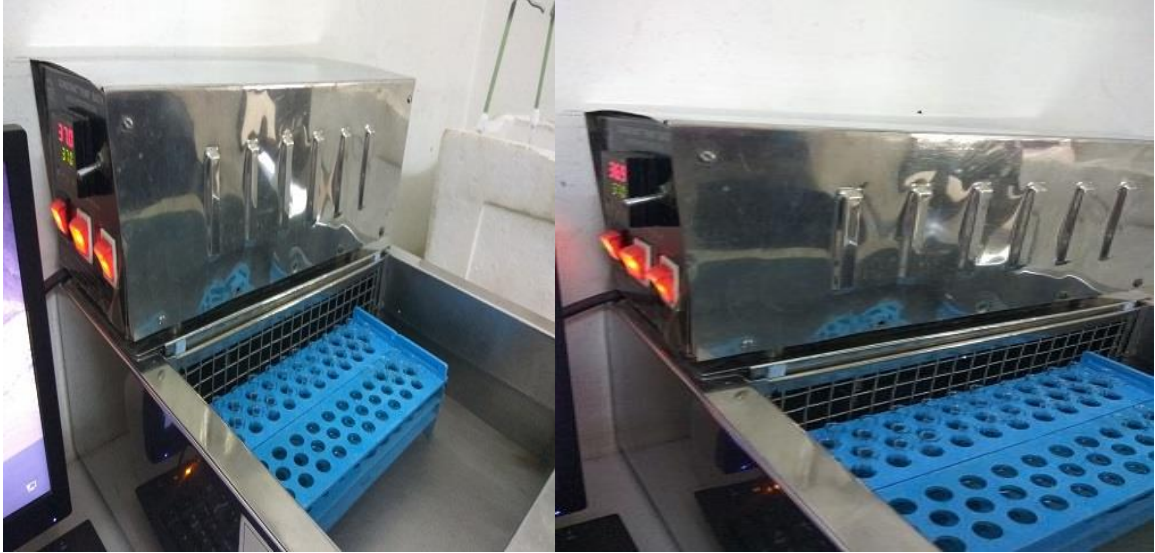


Fig. 3.1 Constant temperature bath

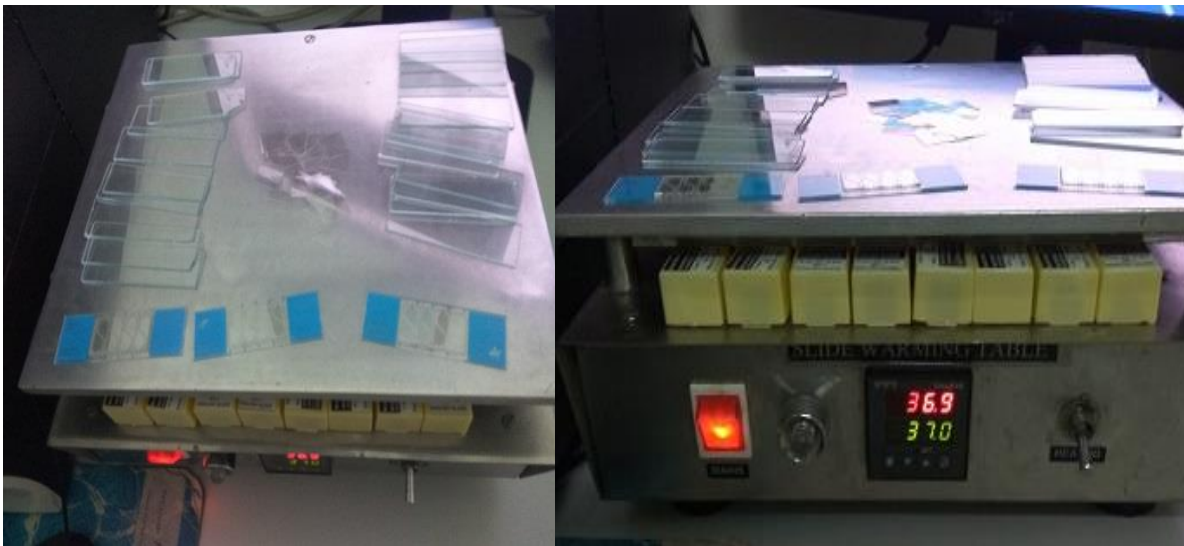


Fig. 3.2 Slide warming table

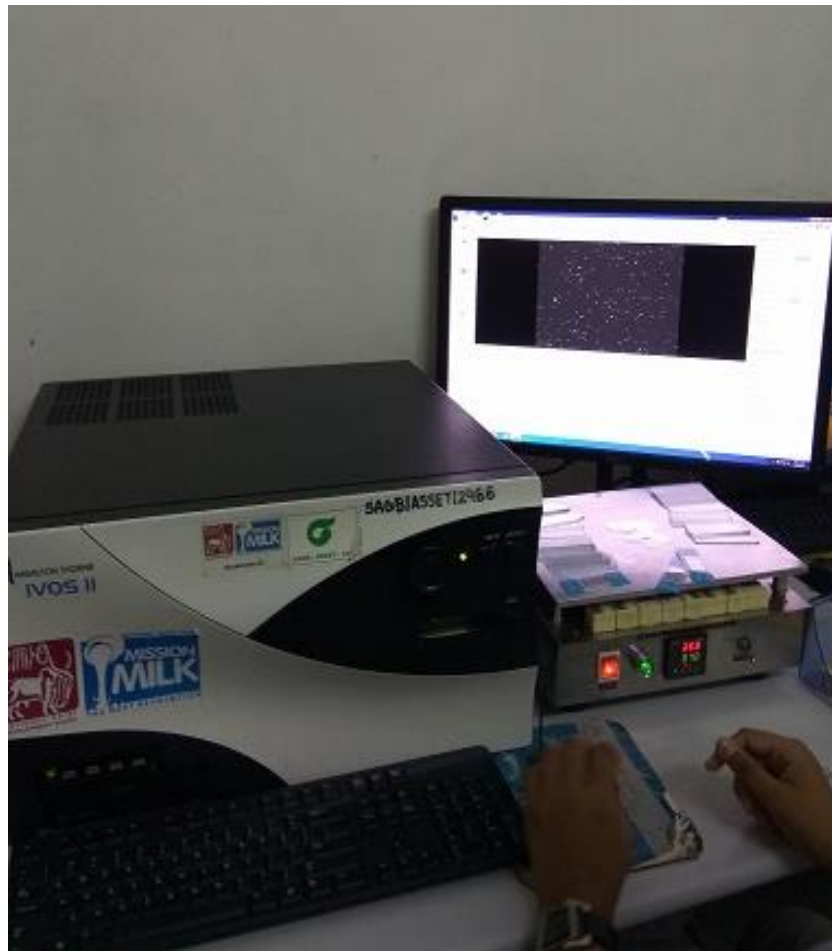


Fig. 3.3 Computer Assisted Sperm Analyzer (Hamilton-Thorne IVOS II)

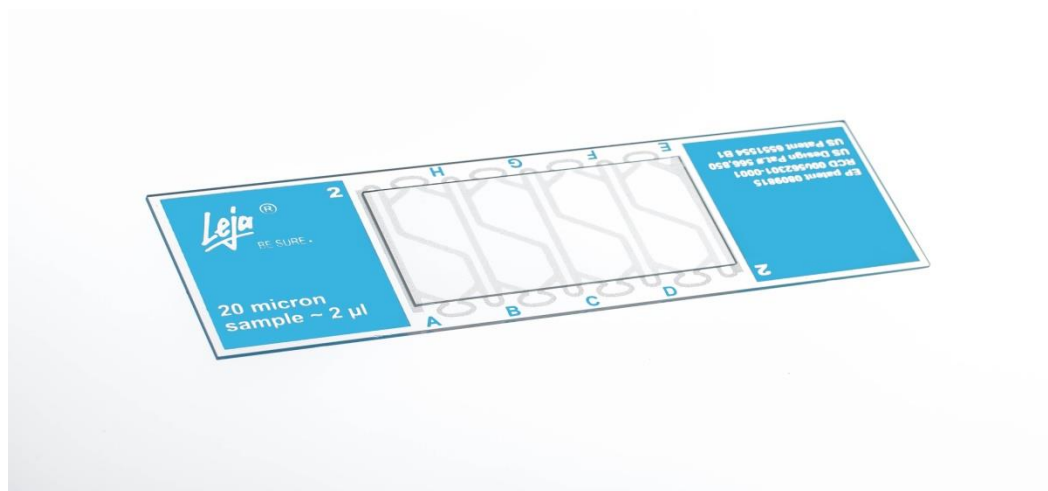


Fig. 3.4 Leja slide (8 - chambered)



Fig. 3.5 Micro – pipette



Fig. 3.6 Micro tips



Fig. 3.7 Cryocan (LN₂ container)

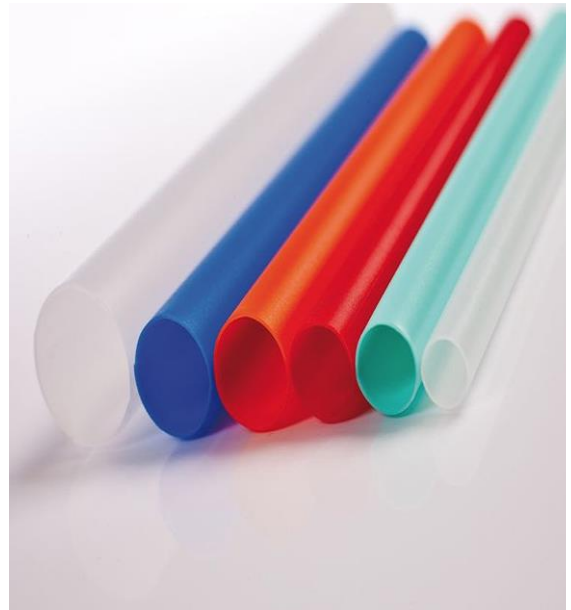


Fig. 3.8 Pencil goblets

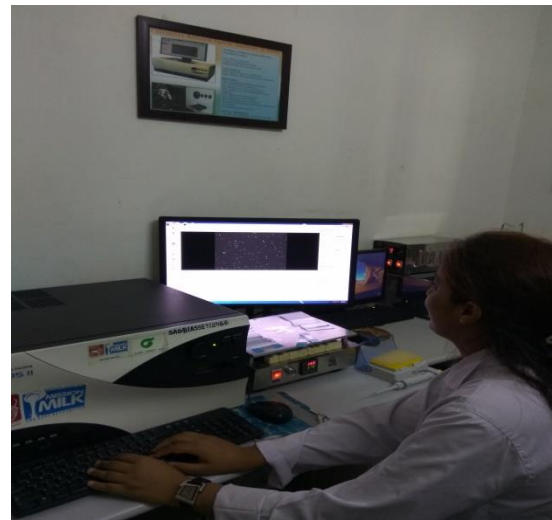


Fig. 3.9 Performing CASA at SAG Unit office, Ahmedabad



*RESULTS
AND
DISCUSSION*

The present research on “Study on Quality of Frozen Semen and Efficiency of AI Technicians and their Impact on Reproductive Performance of Buffaloes under AI Network Programme” was carried out in three districts of Gujarat keeping in view the objectives set in the introduction chapter and experimental design described in chapter of materials and methods. The results obtained has been presented in tabular form as well as in graphical representation and discussed under sub-heads given below:

- 4.1 Profile and efficiency of AI technicians under AI network programme
- 4.2 Computer assisted analysis of frozen-thawed semen
- 4.3 Comparison of profile of various groups of AI technicians with motility and conception rate
- 4.4 Reproductive performance of the buffaloes under AI network programme
- 4.5 Measures to improve AI success rate in the field condition

4.1 Profile and efficiency of AI technicians under AI network programme

4.1.1 Distribution of AI technicians

The random distribution of AI technicians in three districts of Gujarat is shown in **Table 4.1** and **Figure 4.1**. Fourty per cent of AI technicians were randomly selected and from Panchmahal district followed by Sabarkantha (38.55 per cent) and Arvalli (21.69 per cent) were from Sabarkantha and Arvalli.

The highest percentage (43.45 per cent) of the AI technicians were from Himmatnagar followed by Lunawada (36.36 per cent). The AI technicians from Idar and Bayad were 31.25 and 44.44 per cent, respectively.

Table 4.1 Distribution of AI technicians based on selected districts and taluks

Sr. No.	District	Taluka	Total number of AITs	Selected number of AITs	AITs Percent of total
1	Sabarkantha	Himmatngar	19	14	43.45
		Idar	17	10	31.25
		Prantij	4	4	12.50
		Vadali	2	1	3.13
		Talod	3	3	9.38
a			45	32 (38%)*	
2	Arvalli	Bayad	9	8	44.44
		Bhiloda	4	3	16.67
		Modasa	7	5	27.78
		Dhansura	2	2	11.11
b			22	18 (22%)*	
3	Panchmahal	Khanpur	8	6	18.18
		Kadana	9	5	15.15
		Lunawada	16	12	36.36
		Sahera	1	1	3.03
		Kalol	8	2	6.06
		Santrampur	3	2	6.06
		Godhra	4	3	9.09
		Ghoghamba	3	2	6.06
c			52	33 (40%)*	
Total (a+b+c)			119	83	69.75

*Percentage based on total number of selected AI technicians

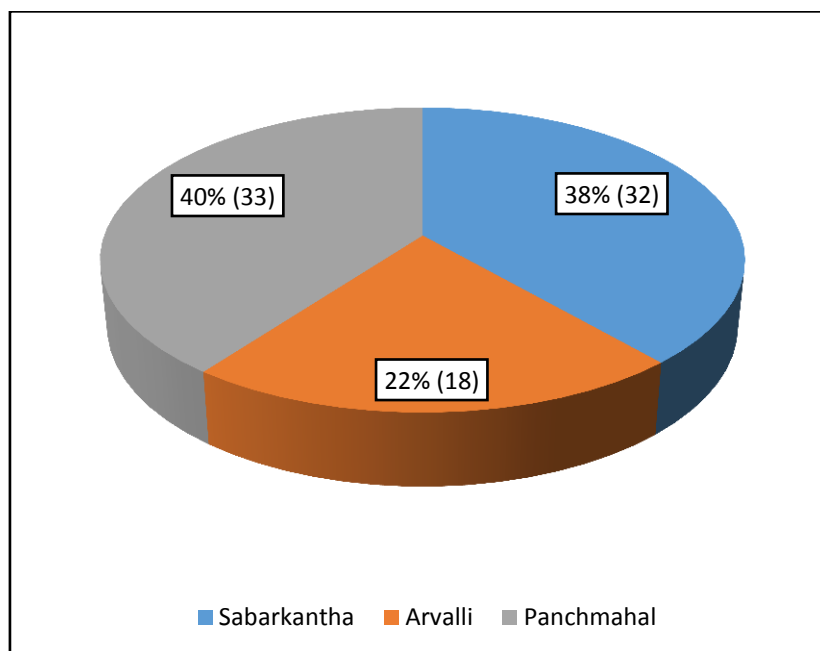


Fig. 4.1 Distribution of AI technicians according to selected districts

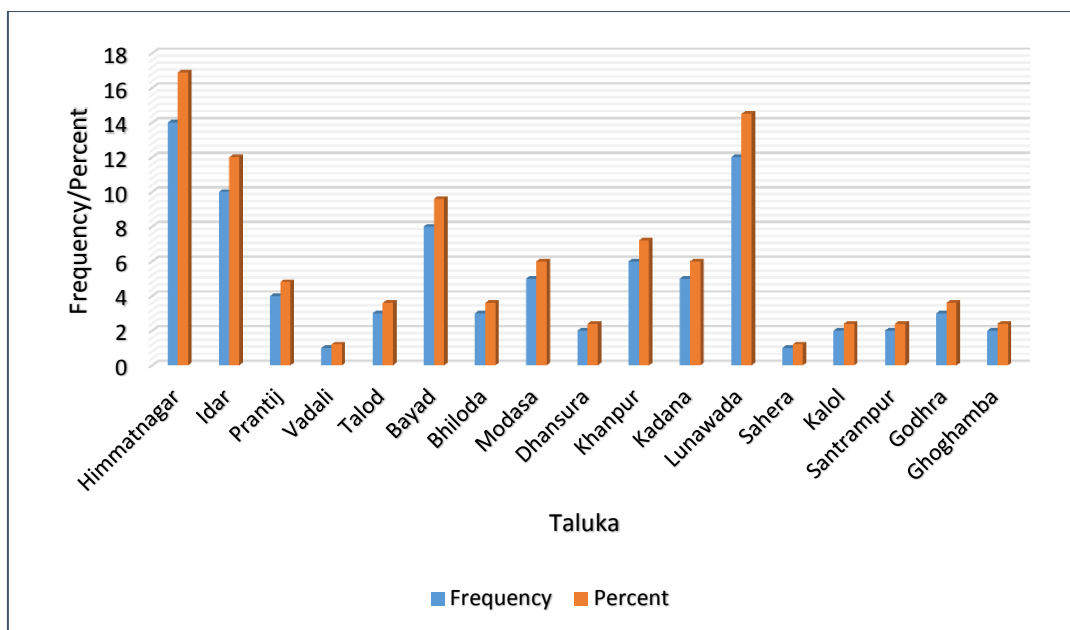


Fig. 4.1a Distribution of AI technicians according to selected taluka

The information on age, level of education, type of AI training, years of experience, time spent for AI activities, estrus detection signs, palpation of ovaries, insemination pattern and thawing of frozen semen straws and insemination practices are given in **Tables 4.2 to 4.12** and **Figures 4.2 to 4.14**.

4.1.2 Age groups of AI technicians

The highest (38.6 per cent) of the AI technicians were from 31 to 40 years of age group followed by 28.90 per cent from 41 to 50 years of age group, 19.30 per cent were from 21 to 30 years of age group and 13.30 per cent were from 51 years of age and above (**Table 4.2; Figure 4.2**).

The observations made in the present study are comparable with the findings of Khokhar (2007) and Nehete (2010) who reported that around 43% of the AITs/respondents were from middle age group followed by 25% and 20% of the respondents were belonging to young and old age groups, respectively.

Table 4.2 Distribution of AI technicians according to their age groups

Sr. No.	Age groups	Number of AI technicians	Percent
1	21 to 30 years	16	19.3
2	31 to 40 years	32	38.6
3	41 to 50 years	24	28.9
4	51 years and above	11	13.3
Total		83	100

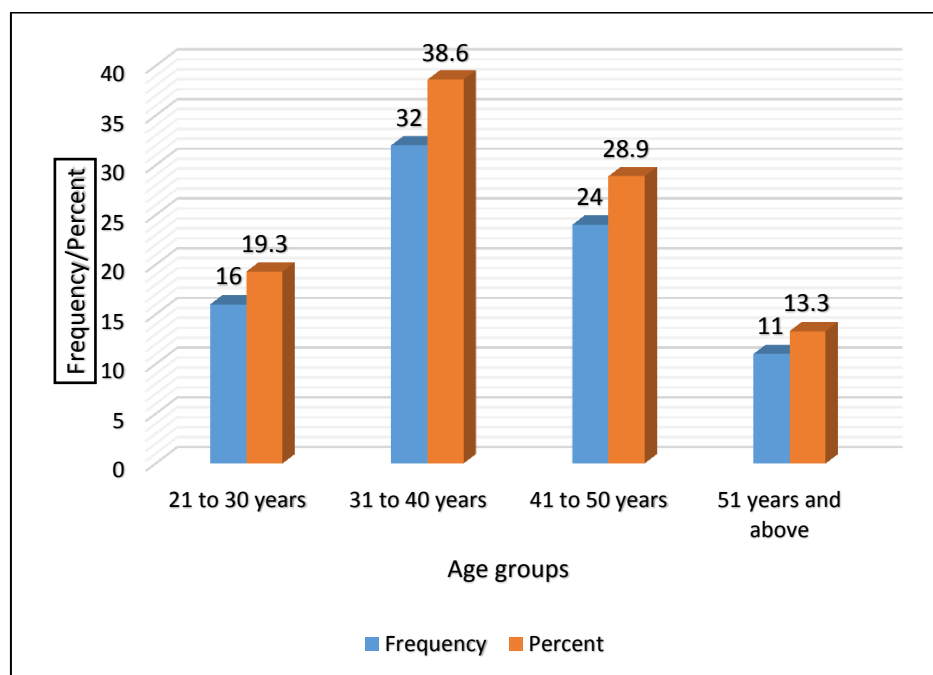


Fig. 4.2 Distribution of AI technicians according to their age groups

4.1.3 Education of the AI technicians

Generally, it is considered that education of the AI technicians play an important role in adoption of scientific practices. Considering these aspects in minds the educational status of AI technicians was studied.

The maximum percentage of the AI technicians have attained education at higher secondary (38.6%) level followed by graduation and above (36.1%) whereas 21.7% of AI technicians have obtained secondary education and only 3.6% of AI technicians were educated below 10th standard. This could be attributed to the availability of secondary and higher secondary schools at the village and taluka level.

Similarly AI technicians also obtained college level education. The higher educational qualifications are preferred for the candidate's selection for AI training due to which level of education among candidates was higher. Moreover, due to rapid development of the educational system in the state/districts, the AI technicians obtained higher education and graduate degrees (Table 4.3; Figure 4.3).

The findings of present study are in harmony with Nehethe (2010) and in partial agreement with study of Temkar (2000) and Khokhar (2007).

Table 4.3 Distribution of AI technicians according to level of education

Sr. No.	Education	Number of AI technicians	Percent
1	Less than 10 th	3	3.6
2	Secondary	18	21.7
3	Higher secondary	32	38.6
4	Graduates and above	30	36.1
Total		83	100

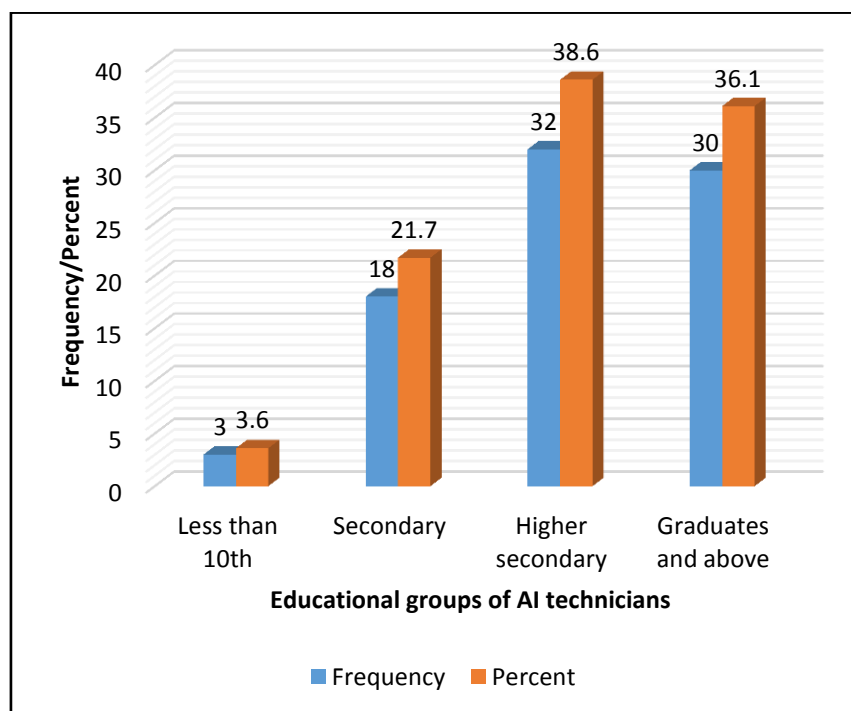


Fig. 4.3 Distribution of AI technicians according to level of education

4.1.4 Types of AI training(s)

Training(s) received by the AI technicians are an important variable contributing to correct technique of insemination that also affects the conception rate in buffaloes.

Prior to employment as an AI technician, each AI technician is provided with a basic training of 4 to 8 weeks by their respective co-operatives and then awarded certificate of successful completion of the AI training. Subsequently they were placed at different AI centers. The duration of refresher training(s) ranges from day 1 to 7.

Majority of the AI technicians have obtained basic training except one. While 77.10 per cent AI technicians have obtained both (basic as well as refresher) type of trainings (**Table 4.4; Figure 4.4**).

The present findings corporate well with the study of Akhtar *et al.* (2007) who reported that most of the AI technicians had obtained one basic training seminar.

Higher AI success rate was observed by Boettcher and Perera (2007) in those technicians who have received refresher trainings.

For the success of AI programme, AI technique and skills are essential. Contrary to this Sutthianlal (2010) reported that 40.38% of the AI technicians had received trainings more than one time whereas 38.47% had received no training.

Table 4.4 Distribution of AI technicians as per the type of training(s) received

Groups	Type of training(s)	Number of AI technicians	Percent
I	Basic	18	21.7
II	Refresher	1	1.2
III	Both	64	77.1
Total		83	100

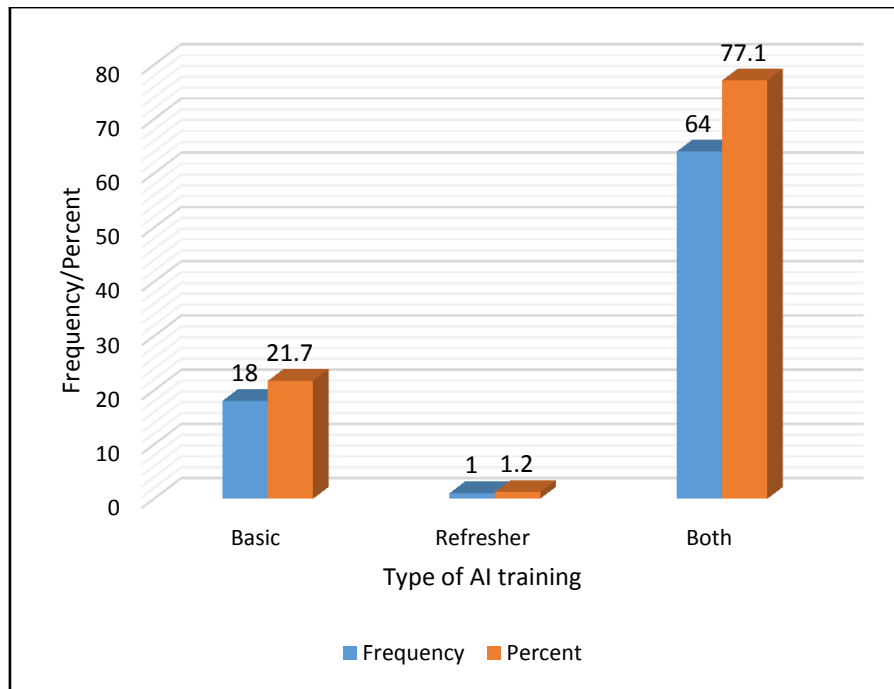


Fig. 4.4 Distribution of AI technicians based on type of AI training(s) received

4.1.5 Experience as an inseminator

The highest percentages (22.9%) of AI technicians are from experience groups I and V followed by group IV (20.5%), group III (19.3%) and group II (14.5%). These data revealed that the maximum numbers of AI technicians are from experience group I who are having experience upto 5 years and group V who were having experience of 21 years and above (Table 4.5, Figures 4.5 and 4.5a).

Studies conducted on profile and practices of AI technicians have shown that experienced technicians have higher success rate than those of new technicians (Everett and Bean, 1986; Adrian *et al.*, 2017). Dixit *et al.* (2016) reported significant gain in skill of the respondents who had undergone training.

AI technician profile parameters have an influence on the success rate of AI. Therefore to improve the national success rate of AI (Adrian *et al.* 2017). Therefore,

to improve the national success rate of AI, the profile, practices and performance of AI technicians to be assessed to maintain a sustainable dairy industry.

Dalton *et al.* (2004) observed that the time duration between thawing and deposition was 4.2 ± 0.17 min and 5.8 ± 0.22 min, respectively, for technicians and breeders. The lack of inseminator experience contribute to an increase in this time.

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).

Other experiences

The evaluation of the profile of the AI technicians revealed that 89.20 per cent of the AI technicians were involved in pregnancy diagnosis in the field whereas 10.80 per cent of AI technicians have performed gynecological and obstetrical work viz., handling of dystocia, torsion, prolapse, etc. Moreover they are expected to do vaccination, deworming and primary health treatment and thereby they assist the veterinarians in field.

Table 4.5 Distribution of AI technicians based on their experience as inseminators

Groups	Experience groups	Number of AI technicians	Percent
I	Up to 5 years	19	22.9
II	6 to 10 years	12	14.5
III	11 to 15 years	16	19.3
IV	16 to 20 years	17	20.5
V	21 years and above	19	22.9
Total		83	100

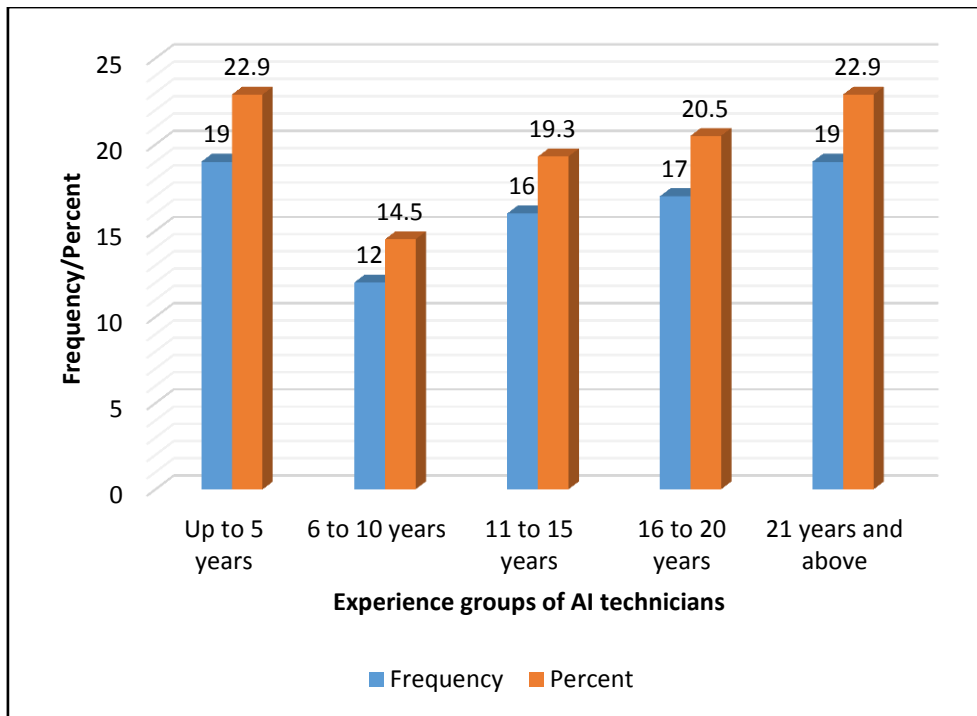


Fig. 4.5 Distribution of AI technicians based on their experience groups

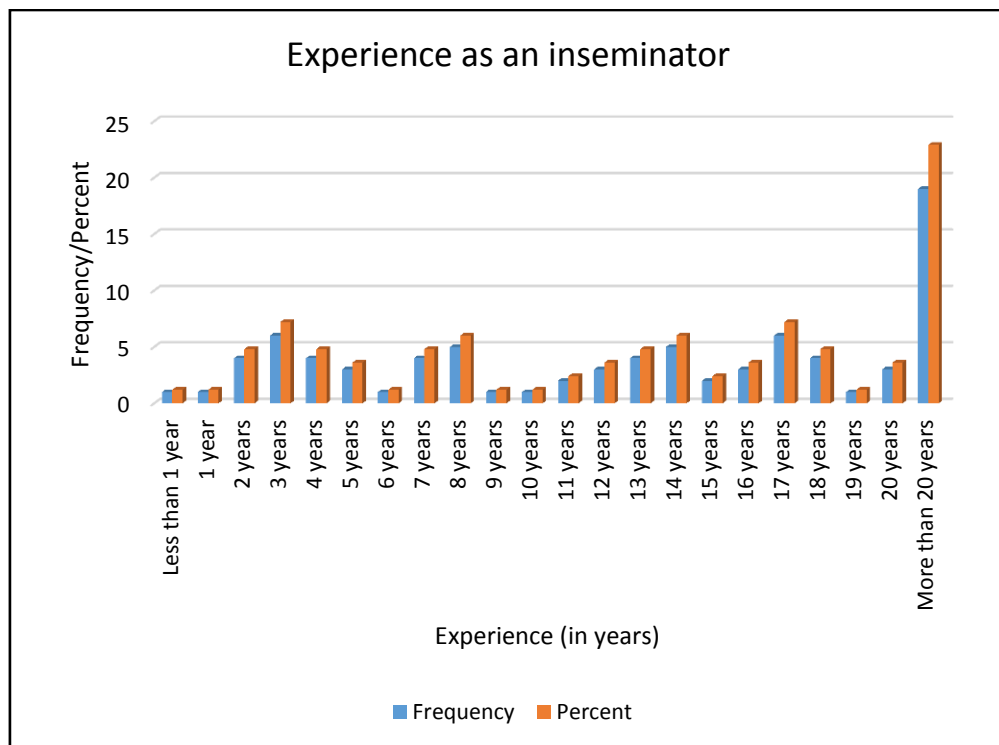


Fig. 4.5a Distribution of AI technicians based on their experience as an inseminator

4.1.6 Signs of estrus detection

The success of Artificial Insemination (AI) depends on critical observation of heat at appropriate time as late insemination leads to failure of conception. Heat detection is the key in the success of an effective breeding program which is achieved by close observation, timed A.I. and sound record keeping (Roelofs *et al.*, 2010).

It was a normal practice by all the AI technicians to check heat prior to perform AI. All the AI technicians have detected the estrus by observing clear mucus discharge. The frequency of other signs of heat detection *i.e.* mounting, mooing/bellowing, swollen vulvar lips and reddening of vulvar mucosa was observed by 31, 35, 31 and 32 AI technicians, respectively. The frequencies of visual observations were also used by AI technicians for heat detection. The unobserved signs of estrus were restlessness (72), mounting (52), bellowing (48), swollen vulvar lips (52) and reddening of vulvar mucosa (51) by the AI technicians (**Table 4.6, Figure 4.6**).

Table 4.6: Signs of estrus detection

Sr. No.	Heat signs	Observed	Unobserved	Total
1	Restlessness	11	72	83
2	Mounting	31	52	
3	Clear vaginal discharge	83	00	
4	Mooing/bellowing	35	48	
5	Swollen vulvar lips	31	52	
6	Reddening of vulvar mucosa	32	51	

Reproductive management is an economic determination in the success of any dairy enterprise. Kumaresan *et al.* (2001) reported that 11.05% of cattle and 20.75% buffaloes are inseminated at proper time. Inadequate heat detection has been identified as a major limit to herd's reproductive performance.

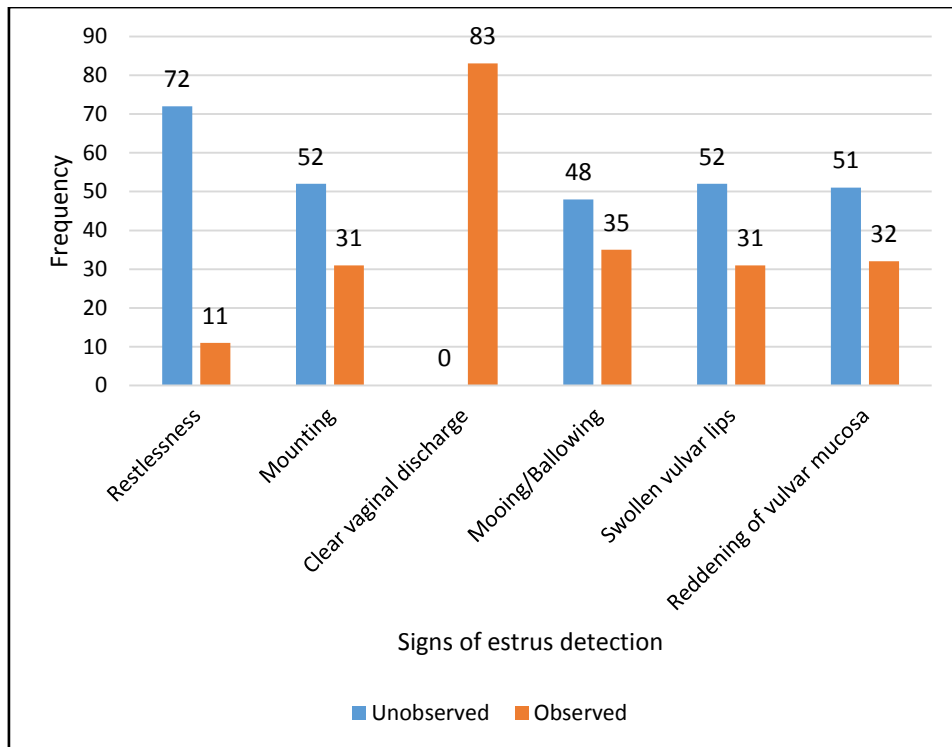


Fig. 4.6 Signs of estrus detection

Thakur *et al.* (2013) reported that visual observation is the best method of heat detection for improving efficiency of estrus detection. They further stated that estrus detection alone contributes considerably to reproductive status of the herd. In present study, the visual observations were used for heat detection by the AI technicians.

In present study, clear vaginal discharge was observed by majority of the AITs as prominent sign of estrus whereas Thakur *et al.* (2013) found standing to be ridden is the best reliable sign of estrus. Ovulation time is well estimated by standing heat. They also observed that visual observation of estrus is the best method for improving efficiency of heat detection.

4.1.7 Time of insemination post estrus:

Highest percentage (50.6%) of AI technicians has performed inseminations between 12 to 18 hours post estrus. Whereas 28.9% and 20.5% of AI technicians have performed inseminations between 6 to 12 hours and 18 to 24 hours post estrus, respectively (Table 4.7; Fig. 4.7).

Timings of AI relative to first detection of heat is known to be critical for achieving high conception rate (Peters, 2005).

Roelofs *et al.* (2005) also indicated the ideal timing for insemination between 12 to 18 hours after onset of estrus. In present study, 50% of the AI technicians have performed insemination between 12 to 18 hours, resulted in high conception rate (**Table 4.7 and 4.21**).

Contrary to our findings, Adrian *et al.* (2017) reported 70% inseminations within 12 hours after exhibition of heat.

Similarly, Graves *et al.* (1997) found no significant difference in AI of jersey cows and heifers once daily or using AM PM rule. They further cautioned that once daily AI doesn't mean once daily estrus detection. Cows and heifers should still be watched for estrus 2-3 times daily for 15 to 20 minutes per watch.

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).

Foote (1979) reported the best time for AI of dairy cows was mid-morning. A single mid-morning AI for all cows first observed in estrus the night before (p.m. – a.m.) for the same morning (a.m. – a.m.) should yield maximum conception.

Table 4.7: Time of insemination post estrus

Sr. No.	Time of insemination post estrus	Number of AI technicians	Percent
1	6 to 12 hrs	24	28.9
2	12 to 18 hrs	42	50.6
3	18 to 24 hrs	17	20.5
Total		83	100

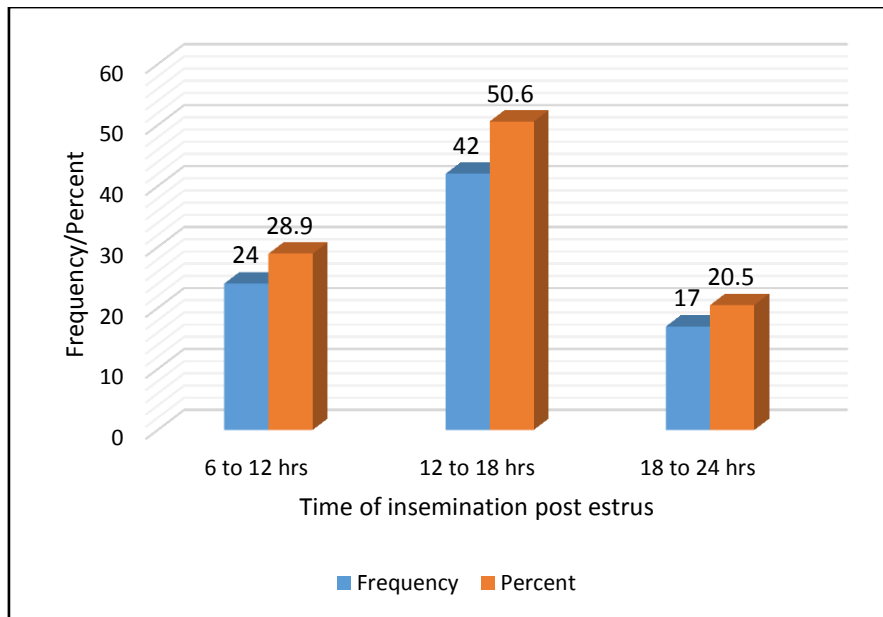


Fig. 4.7 Time of insemination post estrus

4.1.8 Site of insemination in female reproductive tract:

Highest percentage of AI technicians (65.1%) have deposited semen in mid cervix followed by utero-cervical junction (28.9%) and uterine body (6 %) during their regular AI practice (**Table 4.8, Figure 4.8**).

On contrary to our findings, Adrian *et al.* (2017) reported highest percentage (86%) of AI technicians have deposited semen in body of uterus and mid cervix.

It is known that site of semen deposition at entrance, deposition at uterine body and deposition at mid-cervix may also influence on the success of AI.

Souames *et al.* (2015) revealed that inseminators preferred depositing semen in the uterine body than its placement in the uterine horns (74 vs 15%). This was due to the training they have received, in which they were taught the uterine body is the ideal insemination site. In contrast to the present observation of mid-cervix AI, several studies have reported significant increases in conception rates during cornual inseminations compared to the uterine body, such as (64.6 vs 44.7%) by Senger *et al.* (1988) and (30 vs 19%) by Mc Kenna *et al.* (1990). However, no significant

difference was observed in conception rates between cornual inseminations and those performed in the uterine body (Mormont *et al.*, 1989). Nevertheless, specialized training in the technique of deep AI is necessary (Lopez-Gatius, 2000). On contrary to above, Foote (2013) found no effect of semen deposition site on success rate and conception rate.

Table 4.8: Site of insemination in female reproductive tract

Sr. No.	Site of insemination	Number of AI technicians	Percent
1	Mid cervix	54	65.1
2	Utero-cervical junction	24	28.9
3	Uterine body	05	6.0
Total		83	100

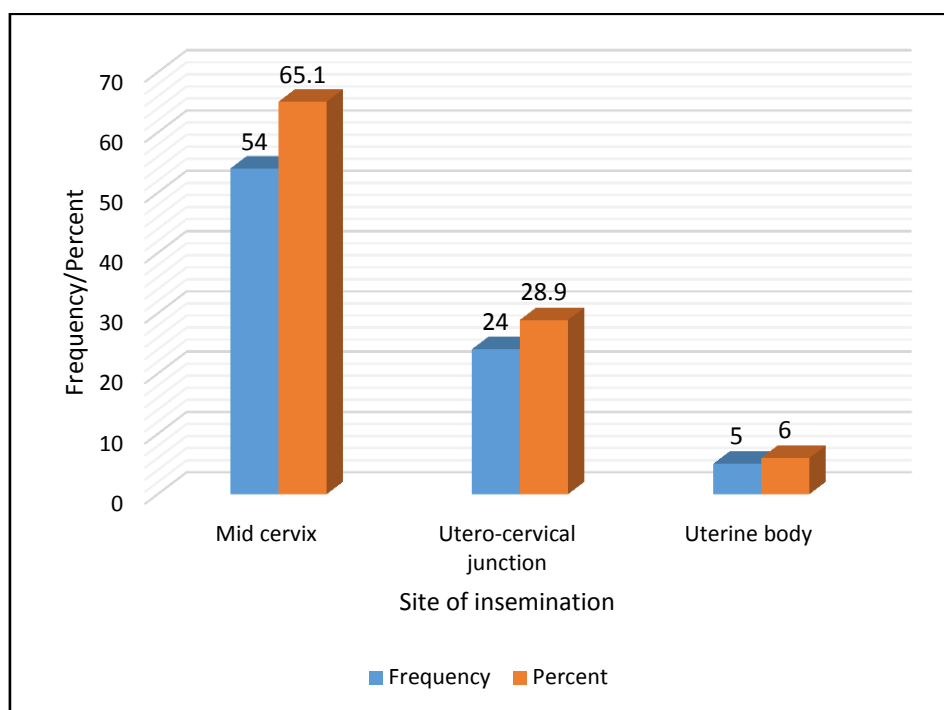


Fig. 4.8 Site of insemination in female reproductive tract

Zobel *et al.* (2011) revealed that the site of semen deposition have influence on calves' sex. But another study noted that semen deposition sites have no effect and that sperm concentration appeared to be more significant in the success of conception (Foote, 2013).

4.1.9 Duration between two inseminations

Highest percentage (60.2%) of the AI technicians have performed single insemination whereas 27.8% of the AI technicians have performed two inseminations at the interval of more than 12 hours. Lowest percentage of the AI technicians have performed inseminations between 6 to 9 hours (2.4%) and 9 to 12 hours (9.6%) (Table 4.9; Figure 4.9).

Table 4.9: Duration between two inseminations

Sr. No.	Duration between two inseminations	Number of AI technicians	Percent
1	Single insemination	50	60.2
2	6 to 9 hours	02	2.4
3	9 to 12 hours	08	9.6
4	More than 12 hours	23	27.8
Total		83	100

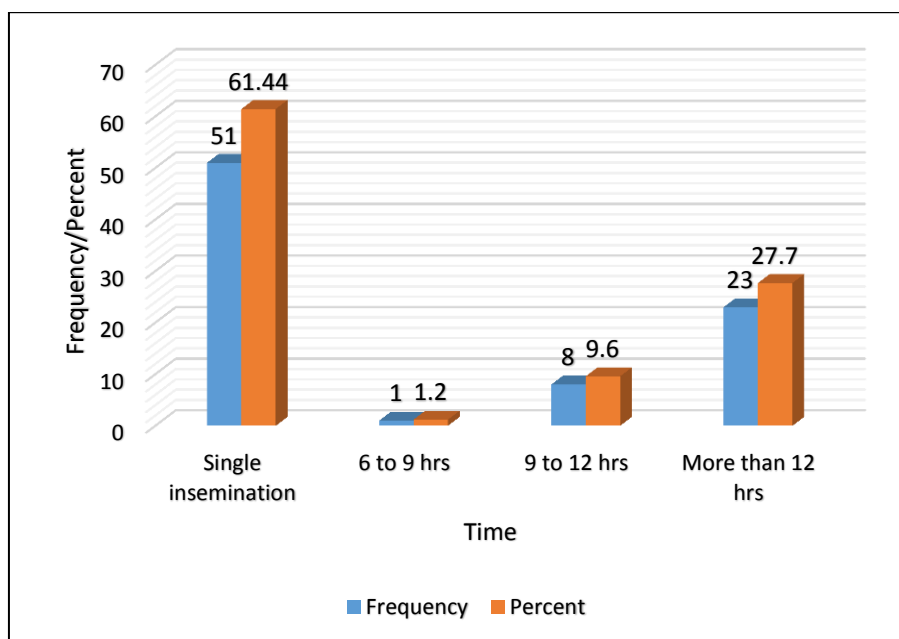


Fig. 4.9 Duration between two inseminations in existing estrus

Present findings revealed that single insemination was preferred by the animal owners and AI technicians as compared to two inseminations at varying interval.

Insemination at optimum time is important for success of AI than the number of times AI performed.

On contrary to present findings, Adrian *et al.* (2017) reported higher percentage (74%) inseminations thrice in estrus followed by 16% twice and 9% once insemination during estrus period in buffaloes. In Philippines water buffalo, this practice is adopted to improve the declining water buffalo population. So that the increased frequency of insemination would result in higher conception rate.

4.1.10 Thawing (temperature and time) of frozen semen straws

Highest percentage (53%) of the AI technicians has thawed semen straws at 37 °C for 30 seconds followed by 3.6% and 1.2% of the AI technicians have thawed semen at 36 °C and 1 minute and 37 °C and 1 minute, respectively. However, 42.2% of the AI technicians were using different thawing temperature and time than the above (**Table 4.10; Figure 4.10**).

In the present study, higher percentage of AI technicians have used thawing temperature and time (37°C and 30 sec.) as they were imparted training to use this temperature and time combination at the respective cooperatives. However, greater number of AI technicians did not follow thawing temperature and time combinations, although they were trained for that.

The process of thawing bovine semen can also have significant effect on fertility rate. Therefore thawing protocol is important for success of AI and it is to be made mandatory for the AI technicians.

Table 4.10: Thawing temperature and time

Sr. No.	Thawing temperature and time	Number of AI technicians	Percent
1	37 °C and 30 sec	44	53
2	37 °C and 1 minute	01	1.2
3	36 °C and 1 minute	03	3.6
4	Any other	35	42.2
Total		83	100

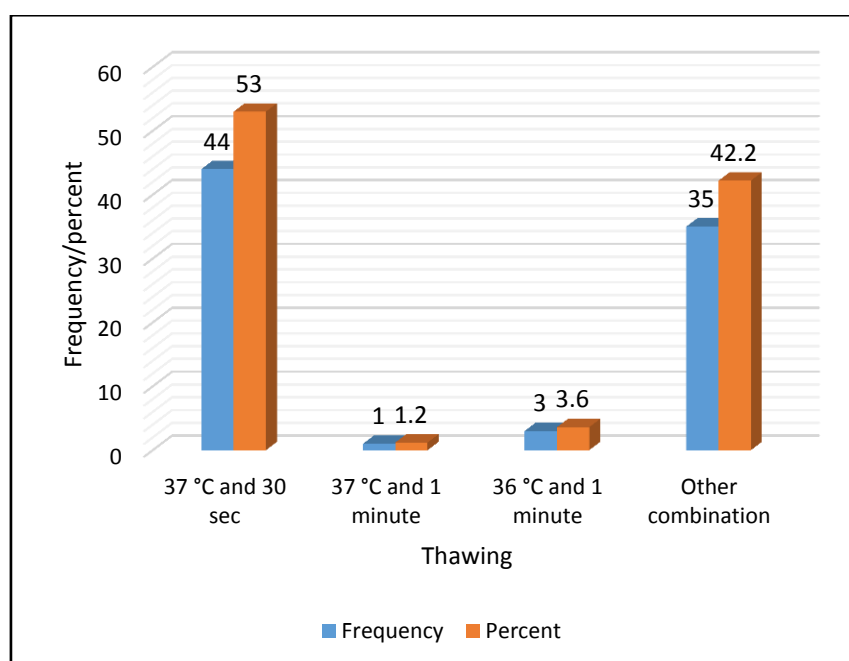


Fig. 4.10 Thawing temperature and time of frozen semen straws

However, Souames *et al.* (2015) reported wide range of thawing of semen in warm water (35-38 °C for 40 seconds) which is most commonly practiced in Algerian cattle. The variations in thawing temperature and time range were greater than the present study.

4.1.11 Cleanliness of instruments

The AI technicians working in three districts have observed good to moderate practices of cleanliness of instruments pertaining to AI (**Table 4.11; Figure 4.11**).

The cleanliness practice was graded in three grades as good, moderate and poor. Out of 83 AITs the good, moderate and poor cleanliness practice was observed

by 64, 18 and 1 AITs, respectively. Similarly, 45 of 83 and 38 of 83 AI technicians have cleaned the forceps and they are graded as good and moderate, respectively.

The dairy cooperatives who train these AITs give emphasis on proper cleanliness practices while AI in the animals. It is very much essential to perform AI in clean and hygienic environment for the satisfactory breeding practices.

Table 4.11: Cleanliness of instruments

Sr. No.	Grades	AI gun	Forceps
1	Good	64	45
2	Moderate	18	38
3	Poor	01	00
Total		83	83

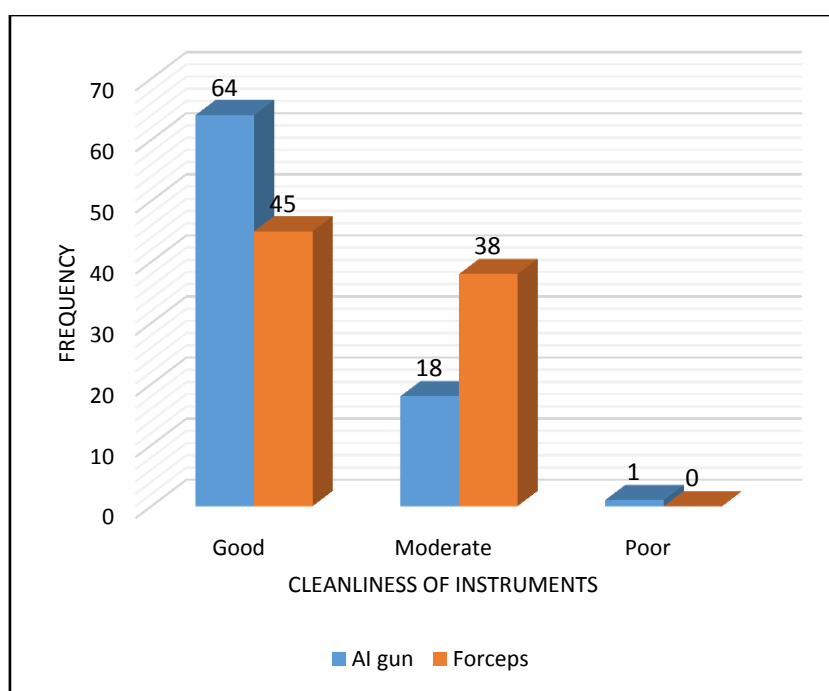


Fig. 4.11 Cleanliness of instruments

4.1.12 Time spent in AI activities by the AI technicians

Highest percentage (84.3%) of AI technicians have spent upto 6 hours per day in AI activities while remaining AI technicians have spent 13.3 per cent of spent 7 to 11 hours a day and only 2.4 per cent have spent 12 to 15 hours a day. All the tasks related to AI activities were performed at the AI centers in morning and

afternoon hours. Similarly the doorstep services to the animal owners were also imparted by the AI technicians. It is mandatory for the AI technicians to maintain various records of reproduction and breeding of the animals. AI technicians are assorted to nearby villages AI center to perform AI at farmers' doorstep. So majority of AI technicians have completed their activities up to 6 hours per day (**Table 4.12; Figure 4.12**).

Table 4.12: Time spent for AI activities

Sr. No.	Time spent for AI activities (hours/day)	Number of AI technicians	Percent
1	Up to 6	70	84.3
2	7 to 11	11	13.3
3	12 to 15	02	2.4
Total		83	100

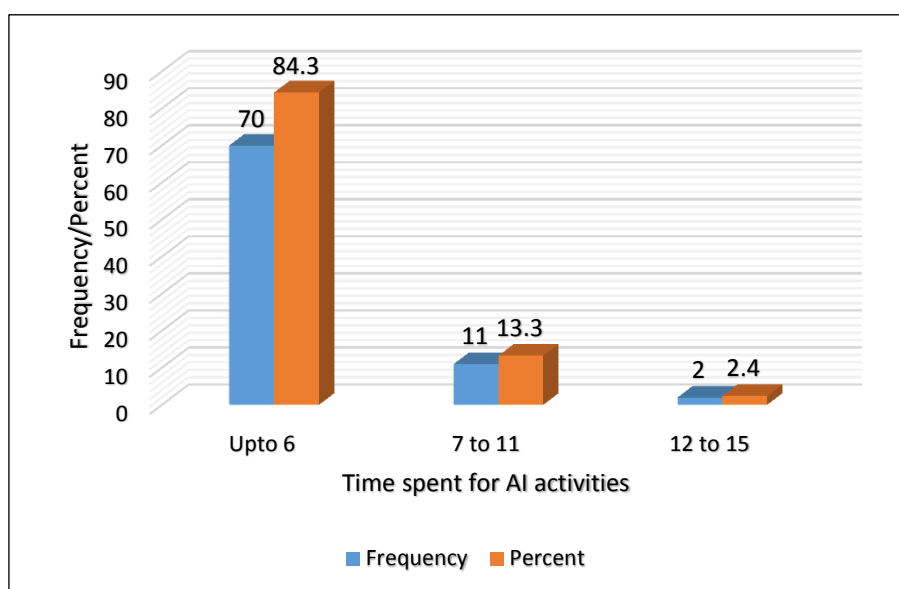


Fig. 4.12 Time spent for AI activities

4.1.13 Ovarian structure (Follicle/Corpus Luteum)

AI technicians from all three districts have common practice to perform ovarian palpation to determine the follicle on the ovary for detection of the heat and subsequent AI of them. In the present study, 74.7 % of AI technicians have performed ovarian palpation for further confirmation of detected heat by palpation of follicles on

ovary. Whereas 25.3% of AI technicians did not perform ovarian palpation. However, they have performed rectal palpation to know the tonicity of uterus as a sign of estrus.

Our findings are in collaboration with findings of Souames *et al.* (2015). They found 89% of the inseminators who have examined reproductive tract at the time of AI in order to decide insemination of cattle when they are truly in heat and exclude those who are not ready for AI.

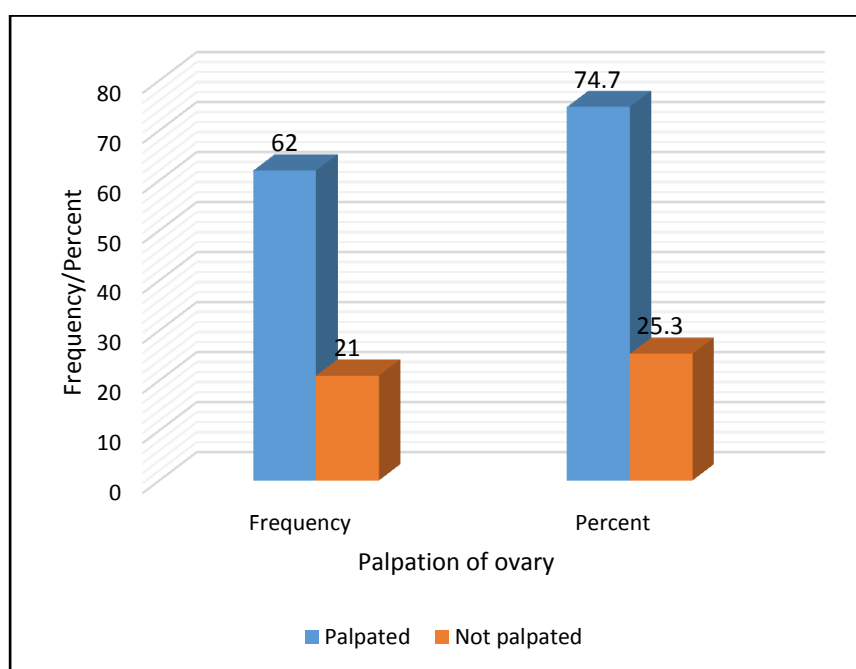


Fig. 4.13 Palpation of ovarian structure

All the breedable animals of the present study under AI network programme were inseminated by AI technicians. However, 21.7% of AI technicians have suggested the animal owners to breed their estrus buffaloes by natural service (**Figure 4.14**). The advice to breed their animals by natural service was given by AI technicians mainly in infertile and repeat breeder animals.

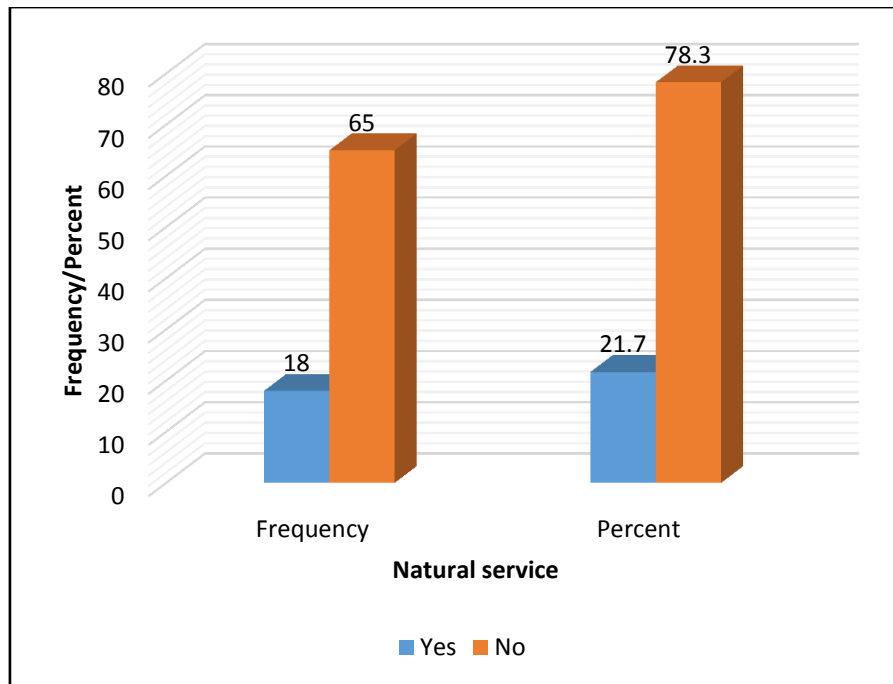


Fig. 4.14 Natural service followed by Artificial Insemination(s)

4.2 Computer Assisted Analysis of Frozen-Thawed Semen

Semen quality evaluation is the most important part in predicting fertility of domestic animals. Due to the complexity of the fertilization process, a single semen evaluation test is not able to predict fertility. Instead, a set of semen tests has to be selected with high relevance for important sperm traits and low redundancy of assay results (Verstegen *et al.*, 2002).

VCL (curvilinear velocity) ($\mu\text{m/s}$) is time-average velocity of a sperm head along with its actual curvilinear path, as perceived in two dimensions in the microscope. VSL (straight line velocity) ($\mu\text{m/s}$) is time-average velocity of a sperm head along the straight line between its first detected position and its last. VAP (average path velocity) ($\mu\text{m/s}$) is time-average velocity of a sperm head along its average path. This path is computed by smoothing the actual path.

BCF (beat cross frequency) (Hz) is the average rate at which the sperm's curvilinear path crosses its average path. While LIN (linearity) is the linearity of a curvilinear path and STR (straightness) is the linearity of the average path.

The overall mean values of velocity ($\mu\text{m/s}$) of post-thawed spermatozoa of samples were: Average Path Velocity (VAP) 100.86 ± 1.97 , straightline velocity (VSL) 86.70 ± 1.76 and curvilinear velocity (VCL) 157.10 ± 3.13 . Further, the mean values of other velocity parameters of post-thawed sperms of samples were: ALH $5.98 \pm 0.13 \mu\text{m}$, BCF $35.53 \pm 0.34 \text{ Hz}$, Straightness 82.66 ± 0.44 per cent, Linearity 55.48 ± 1.42 per cent, Elongation 56.23 ± 0.05 per cent and Area $25.86 \pm 0.43 \mu\text{m}^2$ (**Table 4.13**). These values were showing non-significant difference in relation to the age groups of AI technicians ($p > 0.01$).

The mean velocity parameters ($\mu\text{m/s}$) of post-thawed spermatozoa of semen samples collected from group I (Age: 21 to 30 years) AITs were: VAP 101.15 ± 4.87 , VSL 88.17 ± 4.43 and VCL 154.69 ± 9.43 . Further, the mean values of other velocity parameters of post-thawed sperms of samples were: ALH $5.80 \pm 0.36 \mu\text{m}$, BCF $36.35 \pm 0.44 \text{ Hz}$, Straightness 82.87 ± 0.71 per cent, Linearity 55.48 ± 1.42 per cent, Elongation 55.01 ± 1.18 per cent and Area $25.08 \pm 0.77 \mu\text{m}^2$ (**Tables 4.13**).

The mean velocity parameters ($\mu\text{m/s}$) of post-thawed spermatozoa of semen samples collected from group II (Age: 31 to 40 years) AITs were: VAP 103.06 ± 2.42 , VSL 88.17 ± 4.43 and VCL 154.69 ± 9.43 . Further, the mean values of other velocity parameters of post-thawed sperms of samples were: ALH $6.08 \pm 0.18 \mu\text{m}$, BCF $35.48 \pm 0.56 \text{ Hz}$, Straightness 82.87 ± 0.71 per cent, Linearity 54.65 ± 1.05 per cent, Elongation 53.30 ± 0.61 per cent and Area $26.35 \pm 0.58 \mu\text{m}^2$ (**Table 4.13**).

The mean velocity parameters ($\mu\text{m/s}$) of post-thawed spermatozoa of semen samples collected from group III (Age: 41 to 50 years) AITs were: VAP 99.34 ± 3.01 , VSL 84.80 ± 2.75 and VCL 158.79 ± 4.56 . Further, the mean values of other velocity parameters of post-thawed sperms of samples were: ALH $6.24 \pm 0.16 \mu\text{m}$, BCF $35.27 \pm 0.62 \text{ Hz}$, Straightness $81.79 \pm 0.88 \text{ per cent}$, Linearity $52.67 \pm 1.22 \text{ per cent}$, Elongation $53.23 \pm 0.54 \text{ per cent}$ and Area $26.03 \pm 0.87 \mu\text{m}^2$ (**Table 4.13**).

The mean velocity parameters ($\mu\text{m/s}$) of post-thawed spermatozoa of semen samples collected from group IV (Age: 51 years and above) AITs were: VAP 94.59 ± 11.16 , VSL 81.86 ± 9.81 and VCL 140.97 ± 15.50 . Further, the mean values of other velocity parameters of post-thawed sperms of samples were: ALH $5.21 \pm 0.50 \mu\text{m}$, BCF $35.29 \pm 0.95 \text{ Hz}$, Straightness $85.04 \pm 1.53 \text{ per cent}$, Linearity $59.89 \pm 2.03 \text{ per cent}$, Elongation $53.90 \pm 0.81 \text{ per cent}$ and Area $24.38 \pm 1.91 \mu\text{m}^2$ (**Table 4.13**).

Among the velocity parameters, linearity of spermatozoa were showing significantly higher velocity (59.89 ± 2.03) in semen samples collected from group IV (Age: 51 years and above) followed by group I (Age: 21 to 30 years) (55.48 ± 1.42), group II (31 to 40 years) (54.65 ± 1.05) and group III (Age: 41 to 50 years) (52.67 ± 1.22) (**Table 4.13**). The higher percentage of linearity revealed optimal better semen quality of post-thaw spermatozoa. The higher linearity percentage in group IV is attributed to the better handling of semen straws by these technicians. VAP is the most useful semen motility characteristic which has clinical relevance in the prediction of fertility (Nagy *et al.*, 2015).

Table 4.13: Velocity parameters of frozen-thawed spermatozoa in relation to age groups of AI technicians

Age groups (Years)	N	Mean (\pm SE) velocity parameters of frozen-thawed spermatozoa								
		VAP ($\mu\text{m/s}$)	VSL ($\mu\text{m/s}$)	VCL ($\mu\text{m/s}$)	ALH (μm)	BCF (Hz)	STR (%)	LIN (%)	ELG (%)	ARE (μm^2)
I (21 to 30)	9	101.15 ^a \pm 4.87	88.17 ^a \pm 4.43	154.69 ^a \pm 9.43	5.80 ^a \pm 0.36	36.35 ^a \pm 0.44	82.87 ^a \pm 0.71	55.48 ^b \pm 1.42	55.01 ^a \pm 1.18	25.08 ^a \pm 0.77
II (31 to 40)	30	103.06 ^a \pm 2.42	88.40 ^a \pm 2.19	160.68 ^a \pm 3.90	6.08 ^a \pm 0.18	35.48 ^a \pm 0.56	82.51 ^a \pm 0.63	54.65 ^b \pm 1.05	53.30 ^a \pm 0.61	26.35 ^a \pm 0.58
III (41 to 50)	16	99.34 ^a \pm 3.01	84.80 ^a \pm 2.75	158.79 ^a \pm 4.56	6.24 ^a \pm 0.16	35.27 ^a \pm 0.62	81.79 ^a \pm 0.88	52.67 ^a \pm 1.22	53.23 ^a \pm 0.54	26.03 ^a \pm 0.87
IV (51 and above)	7	94.59 ^a \pm 11.16	81.86 ^a \pm 9.81	140.97 ^a \pm 15.5	5.21 ^a \pm 0.50	35.29 ^a \pm 0.95	85.04 ^a \pm 1.53	59.89 ^c \pm 2.03	53.90 ^a \pm 0.81	24.38 ^a \pm 1.91
Overall	62	100.86 \pm 1.97	86.70 \pm 1.76	157.1 \pm 3.13	5.98 \pm 0.13	35.53 \pm 0.34	82.66 \pm 0.44	55.48 \pm 1.42	56.23 \pm 0.05	25.86 \pm 0.43

Means bearing different superscript within the column, differ significantly ($p < 0.01$)

VAP= Average path velocity, VSL= Straight line velocity, VCL= Curvilinear velocity, ALH= Lateral head displacement, BCF=Beat cross frequency, STR=Straitness, LIN= Linearity, ELG= Elongation, ARE= Area

Table 4.14: Motility characteristics of spermatozoa in relation to age groups

Age Groups (Years)	N	Character (Mean \pm SE)	
		Linear motility	Wobbling motility
I (21 to 30)	9	55.47 ^b \pm 1.42	64.58 ^b \pm 1.32
II (31 to 40)	30	54.64 ^b \pm 1.05	62.02 ^a \pm 0.85
III (41 to 50)	16	52.67 ^a \pm 1.22	62.15 ^a \pm 0.94
IV (51 and above)	7	59.88 ^c \pm 2.02	68.49 ^c \pm 1.45
Overall	62	54.85 \pm 0.71	64.13 \pm 0.58

Means bearing different superscript within the column, differ significantly ($p < 0.01$)

The motility characteristics of spermatozoa in relation to age groups are presented in **Table 4.14**. The linear motility (59.88 ± 2.02) and wobbling motility (68.49 ± 1.45) are significantly higher in group IV (Age: 51 and above) than those of group I, II and III the corresponding values were 55.47 ± 1.42 , 64.58 ± 1.32 ; 54.64 ± 1.05 , 62.02 ± 0.85 and 52.67 ± 1.22 , 62.15 ± 0.94 , respectively.

Linear motility parameters of present study were studied by CASA equipment Hamilton-Thorne IVOS II, is the first of its kind and we have made pioneer work. Hence, the review pertaining to this is not available (**Table 4.14**).

The findings of velocity parameters of the present study were higher than those reported by Patel and Dhama (2013) on CASA evaluation of fresh and frozen-thawed semen of Kankrej x HF crossbred bulls at the Patan semen station. The mean values of Amplitude of Lateral Head displacement (ALH, μm), Beat-Cross frequency (BCF, Hz), Straightness (STR, %), Linearity (LIN, %), Elongation (ELG, %) and Area (ARE, μm^2) of fresh and frozen-thawed sperms of Jafarabadi and Mehsana buffalo bulls did not differ significantly between breeds. Further they found comparatively higher mean values of VAP, VCL and head area (ARE) of frozen-thawed sperm in semen of Mehsana than Jafarabadi buffalo bulls than their own studies made in these breeds of buffaloes (Patel and Dhama, 2012). Although there was no significant difference between the mean values of VSL, ALH and STR for two

breeds. These findings are in harmony with the present study. Except LIN, the values of BCF, STR and ELG were statistically non-significant in the present study. However, Patel and Dhama (2014) found non-significant difference in the values of BCF, STR, LIN and ELG in the semen of Jafarabadi and Mehsana buffalo breeds. Further, they found significant reduction in all values of motility and velocity parameters, except lateral head displacement and elongation of frozen-thawed semen in both the breeds. The corresponding values of the present study are comparable with the report of Patel and Dhama (2014).

CASA offers potential of more accurate prediction of fertility compared to the traditional microscopic semen evaluation technique (Dan Das, 1992; Christensen *et al.*, 1999). Several attempts have been made to correlate different motion characteristics of bull spermatozoa with oocyte penetration rate, *in vitro* fertility rate and field fertility (den Daas, 1992; Kathiravan *et al.*, 2008). Budworth *et al.* (1988) observed a significant correlation between ratio and velocity of motile sperm and fertility. In an early experiment using frozen-thawed bull semen, Amann (1989) observed a correlation between motility/velocity characteristics (total motility rate, VCL, VSL, LIN and BCF) and fertility. In contrast to above finding, Amann and Waberski (2014) observed that the evaluation of sperm motion cannot accurately predict the fertilising potential of sperm when used for AI. They, however, recommend reliance on curvilinear velocity (VCL) and straight-line velocity STR (VSL/VAP) for each spermatozoon with categorization into three groups: immotile, VCL near zero; undesirable, low VCL or STR; and satisfactory, VCL and STR meet standards. Farrell *et al.* (1998) reported very high correlation between motility parameters evaluated by CASA on fresh semen and bull fertility with quite good prognostic value (fertility was defined as 59 days of non-return rate). Oliveira *et al.*

(2013) reported high correlation between multiple combinations of CASA parameters and bull fertility.

Mandal *et al.* (2003) reported significant ($P<0.01$) positive relationships between most of the motility characters of fresh semen of Murrah bulls, except for linearity (%) which was found to be non-significant. Functionality of the sperm membrane was significantly ($P<0.01$) and positively correlated with the sperm kinematics (except linearity). Motility, VSL, VCL and VAP, LIN and ALH showed no significant relationships.

The various sperm motion traits (mean \pm SE) assessed through CASA in the present study collaborate well with the reported values of Kumar *et al.* (2015). The VAP, VSL, VCL, ALH, BCF, STR, LIN and ARE were as 94.48 ± 2.87 , 79.07 ± 2.49 , 160.06 ± 0.26 , 6.56 ± 0.43 , 85.06 ± 0.43 , 83.32 ± 0.68 , 52.68 ± 0.96 and 57.47 ± 2.64 .

The motion kinetics of post-thaw spermatozoa reported by Singh and Balhara (2016) were lower for VAP (87.22 ± 2.4), VSL (68.93 ± 1.9) and LIN (45.59 ± 0.4) than the reported values of the present study. However, our findings of VCL (156.52 ± 4.3), ALH (6.80 ± 0.1), BCF (33.98 ± 0.4) and STR (79.21 ± 0.7) are in agreement with study of Singh and Balhara (2016).

Table 4.15: Velocity parameters of frozen-thawed spermatozoa in relation to experience groups of AI technicians

Experience groups (Years)	N	Mean (\pm SE) velocity parameters of frozen-thawed spermatozoa								
		VAP ($\mu\text{m/s}$)	VSL ($\mu\text{m/s}$)	VCL ($\mu\text{m/s}$)	ALH (μm)	BCF (Hz)	STR (%)	LIN (%)	ELG (%)	ARE (μm^2)
I (0 to 5)	9	97.45 ^a \pm 3.24	84.98 ^a \pm 2.77	148.32 ^a \pm 6.74	5.62 ^a \pm 0.30	36.73 ^b \pm 0.51	82.98 ^a \pm 0.58	55.69 ^a \pm 0.98	54.66 ^a \pm 0.01	25.95 ^a \pm 0.60
II (6 to 10)	8	111.89 ^b \pm 3.72	96.84 ^b \pm 3.50	174.84 ^b \pm 8.05	6.38 ^b \pm 0.42	36.29 ^{ab} \pm 1.35	82.70 ^a \pm 1.28	54.36 ^a \pm 2.10	54.35 ^a \pm 0.02	26.26 ^a \pm 1.01
III (11 to 15)	10	102.74 ^a \pm 4.99	88.14 ^a \pm 4.70	160.11 ^a \pm 6.55	6.06 ^b \pm 0.29	35.12 ^a \pm 0.79	82.36 ^a \pm 0.99	54.22 ^a \pm 1.93	53.61 ^a \pm 0.01	26.42 ^a \pm 0.83
IV (16 to 20)	19	99.82 ^a \pm 2.75	84.49 ^a \pm 2.40	157.74 ^a \pm 4.59	6.17 ^b \pm 0.21	34.77 ^a \pm 0.59	81.47 ^a \pm 0.92	53.50 ^a \pm 1.39	53.51 ^a \pm 0.69	25.97 ^a \pm 0.70
V (21 and above)	15	97.17 ^a \pm 5.64	84.32 ^a \pm 5.02	150.06 ^a \pm 8.23	5.68 ^a \pm 0.27	35.70 ^a \pm 0.66	84.24 ^b \pm 0.78	56.82 ^a \pm 1.39	53.34 ^a \pm 0.49	26.46 ^a \pm 0.87

Means bearing different superscript within the column, differ significantly ($p < 0.01$)

VAP= Average path velocity, VSL= Straight line velocity, VCL= Curvilinear velocity, ALH= Lateral head displacement, BCF=Beat cross frequency, STR=Straightness, LIN= Linearity, ELG= Elongation, ARE= Area

The mean velocity parameters ($\mu\text{m}/\text{sec}$) of post-thawed spermatozoa in relation to experience group I (0 to 5 years) of the AITs were: VAP 97.45 ± 3.24 , VSL 84.98 ± 2.77 and VCL 148.32 ± 6.74 . Further, the mean values of other velocity parameters of post-thawed sperms of samples were: ALH $5.62 \pm 0.30 \mu\text{m}$, BCF $36.73 \pm 0.51 \text{ Hz}$, Straightness 82.98 ± 0.58 per cent, Linearity 55.69 ± 0.98 per cent, Elongation 54.66 ± 0.01 per cent and Area $25.95 \pm 0.60 \mu\text{m}^2$ (**Table 4.15**).

The mean velocity parameters ($\mu\text{m}/\text{sec}$) of post-thawed spermatozoa in relation to experience group II (6 to 10 years) of the AITs were: VAP 111.89 ± 3.72 , VSL 96.84 ± 3.50 and VCL 174.84 ± 8.05 . Further, the mean values of other velocity parameters of post-thawed sperms of samples were: ALH $6.38 \pm 0.42 \mu\text{m}$, BCF $36.29 \pm 1.35 \text{ Hz}$, Straightness 82.70 ± 1.28 per cent, Linearity 54.36 ± 2.10 per cent, Elongation 54.35 ± 0.02 per cent and Area $26.26 \pm 1.01 \mu\text{m}^2$ (**Table 4.15**).

The mean velocity parameters ($\mu\text{m}/\text{sec}$) of post-thawed spermatozoa in relation to experience group III (11 to 15 years) of the AITs were: VAP 102.74 ± 4.99 , VSL 88.14 ± 4.70 and VCL 160.11 ± 6.55 . Further, the mean values of other velocity parameters of post-thawed sperms of samples were: ALH $6.06 \pm 0.29 \mu\text{m}$, BCF $35.12 \pm 0.79 \text{ Hz}$, Straightness 82.36 ± 0.99 per cent, Linearity 54.22 ± 1.93 per cent, Elongation 52.61 ± 0.01 per cent and Area $26.42 \pm 0.83 \mu\text{m}^2$ (**Table 4.15**).

The mean velocity parameters ($\mu\text{m}/\text{sec}$) of post-thawed spermatozoa in relation to experience group IV (16 to 20 years) of the AITs were: VAP 99.82 ± 2.75 , VSL 84.49 ± 2.40 and VCL 157.74 ± 4.59 . Further, the mean values of other velocity parameters of post-thawed sperms of samples were: ALH $6.17 \pm 0.21 \mu\text{m}$, BCF $34.77 \pm 0.59 \text{ Hz}$, Straightness 81.47 ± 0.92 per cent, Linearity 53.50 ± 1.39 per cent, Elongation 53.51 ± 0.69 per cent and Area $25.97 \pm 0.70 \mu\text{m}^2$ (**Table 4.15**).

The velocity parameters found significantly higher in group II (111.89 ± 3.72 , 96.84 ± 3.50 , 174.84 ± 8.05 and 6.38 ± 0.42) group I (36.73 ± 0.51) and group V (84.24 ± 0.78) whereas LIN, ELG and ARE were showing non-significant difference ($p > 0.01$) with the experience groups (**Table 4.15**).

Table 4.16: Tail characteristics of spermatozoa in relation to experience groups

Experience Groups (Years)	N	Character (Mean \pm SE)	
		Coiled tail conc.	Coiled tail count
I (0 to 5)	9	$0.66^c \pm 0.12$	$8.11^c \pm 1.46$
II (6 to 10)	8	$0.51^b \pm 0.16$	$6.13^b \pm 1.91$
III (11 to 15)	10	$0.38^a \pm 0.11$	$4.30^a \pm 1.30$
IV (16 to 20)	19	$0.80^a \pm 0.16$	$9.58^c \pm 1.90$
V (21 and above)	15	$0.35^a \pm 0.08$	$4.27^a \pm 0.90$

Means bearing different superscript within the column, differ significantly ($p < 0.01$)

The tail characters of frozen-thawed spermatozoa shown higher significant difference ($p < 0.01$) in group I (Experience: 0 to 5 years) and group II (Experience 6 to 10 years) than group IV (Experience: 16 to 20 years), III (Experience: 11 to 15 years) and V (Experience: 21 years and above). Similarly the coiled tail count was significantly higher in IV (9.58 ± 1.90) followed by group I (8.11 ± 1.46), II (6.13 ± 1.91), III (4.30 ± 1.30) and V (4.27 ± 0.90) (**Table 4.16**).

The velocity parameters of frozen-thawed spermatozoa in relation to education groups of AITs are presented in **Table 4.17**. VAP, VSL, VCL, ALH, BCF, ELG and ARE were showing non-significant difference ($p > 0.01$) in relation to educational groups of AI technicians (**Table 4.17**).

Straightness (STR) of the spermatozoa was found significantly higher ($p < 0.01$) in group I (less than 10th), followed by group II (upto 10th standard), group IV (graduation and above) and group III (upto 12th standard). Similarly linearity (LIN)

was found significantly higher in group I (58.20 ± 3.79) followed by group II (57.31 ± 1.75), group IV (54.87 ± 0.71) and group III (53.37 ± 0.01) (**Table 4.17**).

The velocity parameters of the frozen-thawed spermatozoa of three districts are presented in Table 4.18. Average path velocity (VAP), ELG and ARE were significantly higher in Panchmahal (104.62 ± 4.09), Sabarkantha (54.18 ± 0.01) and Panchmahal (26.89 ± 0.48), respectively. Whereas the other velocity parameters than these were showing non-significant ($p > 0.01$) difference in relation to districts studied.

The velocity parameters of the frozen-thawed spermatozoa in relation to type of training received are presented in **Table 4.19**. VAP, VCL and ALH (114.48 ± 3.35 , 182.56 ± 5.92 and 6.67 ± 0.27) were significantly higher in the semen straws obtained from AITs who received refresher trainings. Whereas ELG (53.72 ± 0.01) was significantly higher in semen straws collected from AITs who received both (basic and refresher) types of trainings. The rest of the parameters given in **Table 4.19** were showing non-significant difference.

Table 4.17: Velocity parameters of frozen-thawed spermatozoa in relation to educational groups of AI technicians

Education groups	N	Mean (\pm SE) velocity parameters of frozen-thawed spermatozoa								
		VAP ($\mu\text{m/s}$)	VSL ($\mu\text{m/s}$)	VCL ($\mu\text{m/s}$)	ALH (μm)	BCF (Hz)	STR (%)	LIN (%)	ELG (%)	ARE (μm^2)
I (Less than 10 th)	3	93.08 ^a \pm 6.77	79.98 ^a \pm 5.63	140.30 ^a \pm 10.58	5.39 ^a \pm 0.57	35.37 ^a \pm 0.71	84.05 ^b \pm 2.84	58.20 ^b \pm 3.79	54.40 ^a \pm 0.02	24.84 ^a \pm 1.75
II (Upto 10 th)	12	102.83 ^a \pm 6.87	89.07 ^a \pm 6.23	154.38 ^a \pm 9.27	5.56 ^a \pm 0.30	36.40 ^a \pm 0.63	83.57 ^{ab} \pm 1.10	57.31 ^b \pm 1.75	53.47 ^a \pm 0.01	26.44 ^a \pm 0.84
III (Upto 12 th)	27	101.11 ^a \pm 2.54	85.84 ^a \pm 2.24	160.54 ^a \pm 4.53	6.22 ^a \pm 0.20	35.07 ^a \pm 0.62	81.69 ^a \pm 0.75	53.37 ^a \pm 1.13	53.70 ^a \pm 0.01	25.99 ^a \pm 0.50
IV (Graduation and above)	20	100.52 ^a \pm 3.00	87.45 ^a \pm 2.65	156.60 ^a \pm 4.96	5.99 ^a \pm 0.19	35.65 ^a \pm 0.48	83.22 ^{ab} \pm 0.43	54.87 ^a \pm 0.71	53.42 ^a \pm 0.01	26.52 ^a \pm 0.72

Means bearing different superscript within the column, differ significantly ($p < 0.01$)

VAP= Average path velocity, VSL= Straight line velocity, VCL= Curvilinear velocity, ALH= Lateral head displacement, BCF=Beat cell frequency, STR=Straightness, LIN= Linearity, ELG= Elongation, ARE= Area

Table 4.18: Velocity parameters of frozen-thawed spermatozoa in relation to selected districts

Districts	N	Mean (\pm SE) velocity parameters of frozen-thawed spermatozoa								
		VAP ($\mu\text{m/s}$)	VSL ($\mu\text{m/s}$)	VCL ($\mu\text{m/s}$)	ALH (μm)	BCF (Hz)	STR (%)	LIN (%)	ELG (%)	ARE (μm^2)
Sabarkantha	21	97.87 ^a \pm 3.05	84.21 ^a \pm 2.65	153.96 ^a \pm 5.86	5.97 ^a \pm 0.23	35.11 ^a \pm 0.64	83.19 ^a \pm 0.78	55.13 ^a \pm 1.23	54.18 ^c \pm 0.01	25.73 ^a \pm 0.57
Arvalli	20	100.09 ^{ab} \pm 2.92	85.87 ^a \pm 2.77	157.64 ^a \pm 4.43	6.12 ^a \pm 0.22	35.37 ^a \pm 0.66	81.85 ^a \pm 0.75	53.26 ^a \pm 1.40	53.58 ^b \pm 0.01	25.95 ^a \pm 0.79
Panchmahal	21	104.62 ^b \pm 4.09	89.99 ^a \pm 3.63	159.72 ^a \pm 5.93	5.85 ^a \pm 0.20	36.11 ^a \pm 0.43	82.89 ^a \pm 0.75	56.09 ^a \pm 1.01	53.04 ^{ab} \pm 0.01	26.89 ^b \pm 0.48

Means bearing different superscript within the column, differ significantly ($p < 0.01$)

VAP= Average path velocity, VSL= Straight line velocity, VCL= Curvilinear velocity, ALH= Lateral head displacement, BCF=Beat cell frequency, STR=Straightness, LIN= Linearity, ELG= Elongation, ARE= Area

Table 4.19: Velocity parameters of frozen-thawed spermatozoa in relation to type of training(s) received by AI technicians

Type of training	N	Mean (\pm SE) Velocity parameters of frozen-thawed spermatozoa								
		VAP ($\mu\text{m/s}$)	VSL ($\mu\text{m/s}$)	VCL ($\mu\text{m/s}$)	ALH (μm)	BCF (Hz)	STR (%)	LIN (%)	ELG (%)	ARE (μm^2)
Basic	13	100.99 ^a \pm 4.34	86.38 ^a \pm 3.90	156.95 ^a \pm 6.45	5.93 ^a \pm 0.23	36.18 ^a \pm 0.29	81.84 ^a \pm 0.78	53.97 ^a \pm 1.07	53.63 ^b \pm 0.01	26.43 ^a \pm 0.57
Refresher	02	114.48 ^b \pm 3.35	95.61 ^a \pm 8.14	182.56 ^b \pm 5.92	6.67 ^b \pm 0.27	37.05 ^a \pm 0.74	81.83 ^a \pm 3.89	54.03 ^a \pm 2.00	50.60 ^a \pm 0.02	26.84 ^a \pm 2.26
Both	47	100.25 ^a \pm 2.29	86.41 ^a \pm 1.76	156.06 ^a \pm 3.67	5.97 ^a \pm 0.15	35.29 ^a \pm 0.43	82.92 ^a \pm 0.52	55.13 ^a \pm 0.88	53.72 ^b \pm 0.01	26.10 ^a \pm 0.44

Means bearing different superscript within the column, differ significantly ($p < 0.01$)

VAP= Average path velocity, VSL= Straight line velocity, VCL= Curvilinear velocity, ALH= Lateral head displacement, BCF=Beat cell frequency, STR=Straightness, LIN= Linearity, ELG= Elongation, ARE= Area

Table 4.20: Motion characters of frozen-thawed spermatozoa in relation to type of training

Motion characters of frozen-thawed spermatozoa	Type of training		
	Basic Mean \pm SE (N=13)	Refresher Mean \pm SE (N=2)	Both Mean \pm SE (N=47)
Coiled tail concentration	0.38 ^a \pm 0.07	1.31 ^b \pm 1.07	0.58 ^a \pm 0.07
Coiled tail count	4.46 ^a \pm 0.83	15.50 ^c \pm 12.50	7.00 ^b \pm 0.86
Coiled tail percent of total	0.49 ^a \pm 0.09	2.75 ^c \pm 2.25	0.78 ^b \pm 0.1
Coiled tail sample (million)	0.08 ^a \pm 0.02	0.28 ^b \pm 0.24	0.12 ^a \pm 0.02
Motile mean elongation	0.55 ^c \pm 0.01	0.47 ^a \pm 0.02	0.56 ^b \pm 0.01
Slow concentration (million/ml)	15.08 ^b \pm 1.01	5.00 ^a \pm 0.67	13.49 ^b \pm 0.76
Slow count	171.31 ^b \pm 10.40	60.50 ^a \pm 9.50	161.85 ^b \pm 8.80
Slow sample (million)	3.31 ^b \pm 0.24	1.10 ^a \pm 0.15	2.96 ^b \pm 0.17
Total concentration (million/ml)	80.31 ^c \pm 4.39	46.86 ^a \pm 0.56	74.96 ^b \pm 2.47
Total count	923.62 ^b \pm 55.66	565 ^a \pm 7.00	900.15 ^b \pm 28.03
Total sample (million/ml)	17.66 ^b \pm 0.97	10.31 ^a \pm 0.12	16.49 ^b \pm 0.54

Means bearing different superscript within the row, differ significantly ($p < 0.01$)

Motion characters of frozen thawed spermatozoa in relation to type of training received by the AI technicians are given in **Table 4.20**.

The mean coiled tail concentration, count, percent and sample were found to be significantly higher in semen samples collected from AITs who received refresher training than those who received both (basic and refresher) the types of trainings and only basic training. This might be due to less number of AITs (N=2) in this group.

Motile mean elongation, slow concentration, slow count, slow sample, total concentration, total count and total sample were found significantly higher from the samples collected from AITs who received basic training. The corresponding values

for these parameters were 0.55 ± 0.01 , 15.08 ± 1.01 , 15.08 ± 1.01 , 171.31 ± 10.40 , 3.31 ± 0.24 , 80.31 ± 4.39 and 923.62 ± 55.66 , respectively.

4.3 Comparison of profile of various groups of AI technicians with motility and conception rates

The profiles of AI technicians of various age groups were studied in relation to post-thaw and progressive motility of semen straws as well as with conception rate (**Table 4.21**).

The profile of various groups of AI technicians (age, experience, trainings, location and level of education) revealed non-significant difference. The corresponding overall mean post-thaw motility and progressive motility percentage of the above profile parameters were 73.59 ± 13.57 and 50.67 ± 1.52 ; 73.58 ± 13.57 and 50.67 ± 11.99 ; 73.59 ± 13.57 and 50.67 ± 11.99 ; 73.58 ± 13.57 and 50.67 ± 11.94 ; 73.59 ± 13.57 and 50.67 ± 11.99 , respectively (**Table 4.21**).

Significantly higher progressive motility was observed in experience group II (6 to 10 years) than those of other experience groups ($p > 0.05$). The conception rate for the above profile groups was significantly higher in group I (46.28 ± 3.29) than other experience groups.

Similarly, significantly higher conception rate (48.37 ± 0.00) was achieved by the AI technicians who received refresher trainings whereas 43.77 ± 0.93 conception rate was achieved by the AI technicians who received both the trainings (basic and refresher) followed by the AI technicians who received only basic training (37.44 ± 3.27) (**Table 4.21**).

Present findings revealed significantly higher conception rate (46.18 ± 0.97) in buffaloes, that was achieved by AITs of Sabarkantha district followed by Arvalli (41.02 ± 0.99) and Panchmahal (40.66 ± 0.99) districts (**Table 4.21**).

Significantly higher conception rate (45.06 ± 1.74) was achieved by the AI technicians who obtained graduation and above level of education (group IV) followed by AI technicians of group III, who obtained education upto 12th standard (43.93 ± 1.51) and group I of AITs who were educated upto 10th standard (43.19 ± 1.92). However, significantly lower conception rate was achieved by those AI technicians who were educated upto 10th standard ($p < 0.01$) (**Table 4.21**).

Motility of spermatozoa is essential for fertilization and is also a significant part of semen quality evaluation as it is commonly regarded as the most important characteristic associated with fertilizing ability of sperm (Freour *et al.*, 2012).

The assessment of sperm motility was solely based on subjective microscopic evaluation of the total proportion of motile sperms (Broekhuijse *et al.*, 2012). But due to large variations in results observed by laboratories, it became necessary to pay more attention to the characterization of sperm movement (Amann and Weberski, 2014).

The post-thaw motility (%) of the present study was higher than the study of Mohan *et al.* (1994) who reported 38.39 ± 1.42 per cent post thaw motility in Murrah bull semen. Similarly, Murugan and Raman (2003) and Singh *et al.* (2002) and Meena *et al.* (2010) also reported lower post-thaw motility than the present study. The corresponding values of the post-thaw motility of their studies were 50.60%, 43.12 ± 1.75 and 55.45 per cent, respectively in murrah buffalo bull semen. The progressive motility of the present study (50.60 ± 1.52) was lower than the study of Khan and Ijaz

(2007) who reported 60.10 ± 1.34 per cent progressive motility in Nili-Ravi buffalo bull semen.

However Muino *et al.* (2008) reported lower progressive motility (44.10 ± 4.75) in buffalo bull semen than the present study.

The higher post-thaw motility of present study is attributed to strict quality control norms of the dispatching center of SAG unit office, Ahmedabad as the post-thaw motility below 50 per cent were rejected and higher post-thaw motile semen straws were made available to field AI centers. Therefore, due to higher post-thaw motility optimum conception rate was achieved by the AITs working under AI network programme.

Table 4.21: Post-thaw and progressive motility of frozen semen straws in relation to AI technicians' profile

Information of AI technicians		Post-thaw motility %	Progressive motility %	Conception rate %
Age groups (Years)	I (21 to 30)	75.55 ^a ± 11.83	51.67 ^a ± 3.29	44.92 ^a ± 3.34
	II (31 to 40)	73.81 ^a ± 14.53	51.73 ^a ± 2.06	43.29 ^a ± 1.34
	III (41 to 50)	71.53 ^a ± 12.01	48.68 ^a ± 2.35	42.49 ^a ± 2.20
	IV (51 and above)	74.79 ^a ± 16.92	49.01 ^a ± 8.24	37.90 ^a ± 2.09
	Overall	73.59 ± 13.57	50.67 ± 1.52	42.68 ± 1.01
Years of experience as an inseminator	I (0 to 5)	71.60 ^a ± 12.20	49.30 ^a ± 10.03	46.28 ^b ± 3.29
	II (6 to 10)	81.23 ^a ± 7.98	58.98 ^b ± 5.53	45.18 ^b ± 2.36
	III (11 to 15)	73.26 ^a ± 15.66	50.77 ^a ± 12.51	40.45 ^a ± 3.13
	IV (16 to 20)	73.45 ^a ± 13.90	49.36 ^a ± 1 0.60	42.80 ^{ab} ± 1.69
	V (21 and above)	71.10 ^a ± 14.95	48.75 ^a ± 15.93	40.62 ^a ± 1.68
	Overall	73.58 ± 13.57	50.67 ± 11.99	42.68 ± 1.01
Type of AI training	Basic	75.87 ^a ± 10.17	51.55 ^a ± 9.80	37.44 ^a ± 3.27
	Refresher	65.25 ^a ± 15.77	48.30 ^a ± 15.27	48.37 ^c ± 0.00
	Both	73.31 ^a ± 14.40	50.53 ^a ± 12.65	43.77 ^b ± 0.93
	Overall	73.59 ± 13.57	50.67 ± 11.99	42.68 ± 1.01
Districts	Sabarkantha	75.96 ^a ± 11.98	51.86 ^a ± 8.99	46.18 ^b ± 0.97
	Arvalli	72.34 ^a ± 17.21	49.65 ^a ± 14.07	41.02 ^a ± 0.99
	Panchmahal	72.67 ^a ± 11.39	50.45 ^a ± 12.93	40.66 ^a ± 0.99
	Overall	73.58±13.57	50.67±11.94	42.68 ± 0.60
Education groups	I (Less than 10 th)	79.90 ^a ± 10.90	50.67 ^a ± 12.38	43.19 ^b ± 1.92
	II (Upto 10 th)	74.03 ^a ± 14.11	50.67 ^a ± 17.41	35.86 ^a ± 1.69
	III (Upto 12 th)	74.83 ^a ± 14.60	51.49 ^a ± 11.03	43.93 ^b ± 1.51
	IV (Graduation and above)	70.70 ^a ± 12.42	49.57 ^a ± 10.07	45.06 ^b ± 1.74
	Overall	73.59 ± 13.57	50.67 ± 11.99	42.68 ± 1.01

Means bearing same superscript within the columns, do not differ significantly (p>0.05)

4.4 Reproductive performance of the buffaloes under AI network programme

Reproduction plays a vital role in animals. Delayed puberty, poor estrus expression, prolonged calving intervals and seasonal breeding limit the reproductive performance.

Age at first AI (months) in buffaloes of Sabarkantha district was lower 35.38 ± 1.13 . The age at first AI for Arvalli and Panchmahal districts were 36.50 ± 1.42 and 37.95 ± 4.97 months, respectively (**Table 4.22**).

The age at first AI of the present study was in agreement with the age at puberty reported by Sompala (2002) who found 24 to 30 months age at puberty in Murrah and crossbred buffaloes. Similarly, Rakshe (2003) reported 16 to 40 months age at puberty in Murrah buffaloes. The age at puberty reported in Nili-ravi buffaloes was 23 to 36 months (Khanum *et al.*, 2012); 30 to 36 months in Surti buffaloes (Sethi, 2003) and 28 to 32 months in Bhadawari breed of buffaloes (Singh, 2000; Sethi, 2003).

The age at first calving (AFC) in buffaloes of Sabarkantha district was lower than the buffaloes of Arvalli and Panchmahal districts. The AFC of buffaloes was 46.29 ± 2.26 , 55.08 ± 3.92 and 55.08 ± 3.92 for Sabarkantha, Arvalli and Panchmahal district, respectively (**Table 4.22**). Borghese *et al.* (2003) reported much lower AFC (*i.e.* 36 months) than the present study. However, AFC in Murrah buffaloes reported by Rakshe (2003) was 37 to 57 months. AFC in Nili-ravi buffaloes was 40 to 42 months (Khanum *et al.*, 2012), was 33 to 56 months in Surti buffaloes (Sethi, 2003) and was 48 to 51 months in Bhadawari breed of buffaloes (Singh, 2000; Sethi, 2003). They found little lower AFC than the present study.

The Carabao buffalo in Philippines had an average age at first calving of 43 months, slightly shorter than river buffalo (45-52 months). The age at first calving for the Murrah x Carabao crossbreed females was recorded at 39 months, which is shorter than both purebreds (Karen *et al.*, 2007).

Buffalo is characterized by delayed puberty, prolonged post-partum ovarian activity and long calving intervals and a tendency for seasonality (Noakes *et al.*, 2001).

The open period in buffaloes of Sabarkantha district was 125.32 ± 2.97 days while this period was similar for Arvalli and Panchmahal districts (156.63 ± 22.4) (**Table 4.22**).

Calving interval in buffaloes of Panchmahal district was 442.89 ± 13.75 days followed by 440.67 ± 4.11 and 423.29 ± 8.97 for buffaloes of Arvalli and Sabarkantha districts, respectively (**Table 4.22**). In contrast to present study, Borghese *et al.* (2011) reported shorter calving interval as short as 400 days in Italian Mediterranean buffaloes the shorter duration was due to better nutritional management of them.

The calving interval in Murrah, Nili-Ravi and Egyptian buffaloes varies from 479 to 508 days (Bhat, 2010). However, Singh (2002a) reported longer calving interval (583 days) in Surti buffaloes than the present study.

A large number of variables govern the calving interval including season of calving, lactation yields and age at first calving (Shah, 2007). The optimum calving interval of buffaloes of Sabarkantha district is attributed to better management of these buffaloes in the district.

Table 4.22: Reproductive performance of buffaloes of selected districts under AI network programme

Districts	Taluka	Age at first AI (months) (N)	Age at First Calving (months) (N)	Open period (days) (N)	Calving interval (days) (N)
Sabarkantha	Himmatnagar	35.90 (435)	47.98 (265)	127.73 (121)	411.61 (62)
	Idar	33.40 (2018)	45.22 (1291)	127.11 (615)	433.41 (272)
	Prantij	36.10 (172)	45.76 (94)	125.27 (37)	424.88 (17)
	Vadali	36.00 (272)	49.09 (160)	121.15 (60)	423.25 (28)
	Talod	35.50 (20)	43.40 (5)	-	-
Mean ± SE (N)		35.38 ± 1.13 (2917)	46.29 ± 2.26 (1815)	125.32 ± 2.97 (833)	423.29 ± 8.97 (379)
Arvali	Bayad	37.40 (191)	52.33 (126)	130.10 (50)	437.87 (24)
	Bhiloda	34.50 (434)	47.02 (282)	140.51 (105)	444.36 (57)
	Modasa	36.50 (231)	48.50 (150)	136.26 (68)	436.41 (31)
	Dhansura	37.60 (43)	52.10 (19)	171 (2)	444 (1)
Mean ± SE (N)		36.50 ± 1.42 (899)	55.08 ± 3.92 (577)	156.63 ± 22.4 (225)	440.67 ± 4.11 (113)
Panchmahal	Khanpur	35.20 (623)	51.79 (362)	140.27 (124)	452.70 (67)
	Kadana	37.10 (307)	51.29 (155)	151.43 (69)	455.84 (32)
	Lunawada	39.60 (1623)	56.15 (951)	142.39 (210)	446.18 (149)
	Sahera	41.10 (254)	60.84 (135)	145.64 (56)	447.13 (29)
	Kalol	40.90 (407)	59.12 (187)	163.53 (30)	437.85 (21)
	Santrampur	27.00 (2)	-	-	-
	Godhra	41.00 (76)	51.04 (32)	148.70 (10)	414.50 (2)
	Ghoghamba	41.70 (118)	55.31 (49)	204.42 (7)	446 (1)
Mean ± SE (N)		37.95 ± 4.97 (3410)	55.08 ± 3.92 (1871)	156.63 ± 22.4 (506)	442.89 ± 13.7 (301)

N = Number of observations

4.5 Measures to improve AI success rate in the field condition

As mentioned earlier in results and discussion, proper estrus detection, using correct thawing temperature and time, timely AI, right deposition of semen in female reproductive tract (either in mid cervix or body of uterus) have influence on success of AI.

Around 30% of the AI technicians of Arvalli district have demanded refresher trainings for the improvement of success rate and other AI practices. AI technicians felt that if pencil goblets are made available to them then they can carry required number of semen straws in the field.

The major constraints reported by AI technicians are:

- Timely replacement of mini LN₂ container for field condition.
- Inadequate supply of protective wears mainly gumboots and hand gloves.
- Non-availability of travis at the doorstep of owner. Hence restraint of animals becomes difficult task for performing AI services.

*SUMMARY
AND
CONCLUSIONS*

The present research “Study on Quality of Frozen Semen and Efficiency of AI technicians and their impact on Reproductive Performance of Buffaloes under AI Network Programme” was conducted in field areas of Sabarkantha, Arvalli and Panchmahal districts of Gujarat and Computer Assisted Semen Analysis (CASA) was done at laboratory situated in SAG, unit office, Ahmedabad with the main objective of ascertaining the efficiency of AI technicians and reproductive performance of buffaloes under AI network programme. It also included the study of post thaw motility of spermatozoa by CASA and suggests remedial measures to improve success rate in the field.

Information on attributes of 83 AI technicians was collected by semi structured questionnaire prepared for personal interview. Apart from this, reproductive data of buffaloes were collected from secondary source *i.e.* INAPH, developed by NDDB Anand.

In total 62 frozen mini straws were collected from 31 randomly selected AI technicians working at the different AI centers of Sabarkantha, Arvalli and Panchmahal districts of Gujarat and CASA was done. These samples were analysed for post-thaw motility, different velocities and sperm abnormalities.

The data on reproductive performance of buffaloes of selected districts were analysed for age at first AI (months), age at first calving (months), open period and calving interval (days).

The information and results of attributes of AI technicians, CASA and reproductive performance of buffaloes are summarized below:

5.1 Profile and Efficiency of AI technicians

The highest percentage of the AI technicians (38.6%) were from 31 to 40 years of age group followed by 28.90 per cent from 41 to 50 years, 19.30 per cent were from 21 to 30 years of and 13.30 per cent were from 51 years of age and above.

The AI technicians who obtained higher secondary education were 38.6% followed by graduation and above (36.1%) and secondary education (21.7%) and only 3.6% of AI technicians were educated below 10th standard.

Majority of the AI technicians had undergone basic training of 2-3 months except for one. While 77.10 per cent AI technicians had undergone both (basic as well as refresher) type of trainings. Training(s) received by the AI technicians were an important variable contributing to correct technique of insemination which also affects the conception rate in buffaloes.

The maximum numbers of AI technicians were from experienced group I with having experience upto 5 years and group V with experience of 21 years and above.

All the AI technicians (n = 83) have detected estrus by observing clear mucus discharge. The frequencies of other signs of heat detection *i.e.* mounting, mooing/bellowing, swollen vulvar lips and reddening of vulvar mucosa observed by AI technicians were 31, 35, 31 and 32, respectively. Optimal conception rate observed in this study was attributed to proper detection of estrus signs by AI technicians.

The ovarian follicles were palpated by 74.7% of the AI technicians for the further confirmation of detected heat, whereas 25.3% of the AI technicians did not perform ovarian palpation but palpated tonicity of the uterus as a sign of estrus.

In the present study, 50% of the AI technicians have performed insemination between 12 to 18 hours, which resulted in significantly higher conception rate (46.18

± 0.97) in buffaloes of Sabarkantha district followed by Arvalli (41.02 ± 0.99) and Panchmahal (40.66 ± 0.99).

Fifty three per cent of the AI technicians have followed standard thawing temperature and time (37°C and 30 seconds). However, 42.2% of the AI technicians did not follow thawing temperature and time combinations, in spite of their training. Very few AI technicians have used different thawing temperature and time.

Highest percentage of AI technicians (65.1%) have deposited semen in mid cervix followed by utero-cervical junction (28.9%) and uterine body (6 %) during their regular AI practice. Majority (60.2%) of the AI technicians have performed single insemination whereas 27.8% of the AI technicians have performed two inseminations at the interval of more than 12 hours.

The AI technicians working in three districts have observed good to moderate practices of cleanliness of instruments pertaining to AI. Majority (84.3%) of AI technicians have spent upto 6 hours per day in AI activities while remaining AI technicians (13.3%) spent 7 to 11 hours a day and only 2.4 per cent have spent 12 to 15 hours a day. It is mandatory for the AI technicians to maintain various records of reproduction and breeding of the animals. AI technicians were assorted to nearby village AI centers to perform AI at farmers' doorstep.

5.2 Computer Assisted Analysis of Frozen-Thawed Semen

The overall mean values of velocity ($\mu\text{m/s}$) of post-thawed spermatozoa of samples were: Average Path Velocity (VAP) 100.86 ± 1.97 , straightline velocity (VSL) 86.70 ± 1.76 and curvilinear velocity (VCL) 157.10 ± 3.13 . Further, the mean values of other velocity parameters of post-thawed sperms of samples were: ALH $5.98 \pm 0.13 \mu\text{m}$, BCF $35.53 \pm 0.34 \text{ Hz}$, Straightness 82.66 ± 0.44 per cent, Linearity 55.48 ± 1.42 per cent, Elongation 56.23 ± 0.05 per cent and Area $25.86 \pm 0.43 \mu\text{m}^2$.

Straightness (STR) of the spermatozoa was found significantly higher ($p < 0.01$) in group I (less than 10th), followed by group II (upto 10th standard), group IV (graduation and above) and group III (upto 12th standard). Similarly linearity (LIN) was found significantly higher in group I (58.20 ± 3.79) followed by group II (57.31 ± 1.75), group IV (54.87 ± 0.71) and group III (53.37 ± 0.01).

The linear motility (59.88 ± 2.02) and wobbling motility (68.49 ± 1.45) were significantly higher in group IV (Age: 41 to 50 years) than those of group I (21 to 30 years), II (31 to 40 years) and III (41 to 50 years), with the corresponding values of 55.47 ± 1.42 , 64.58 ± 1.32 ; 54.64 ± 1.05 , 62.02 ± 0.85 and 52.67 ± 1.22 , 62.15 ± 0.94 , respectively.

The tail characters of frozen-thawed spermatozoa showed higher significant difference ($p < 0.01$) in experienced groups of AI technicians. Significant difference in group I (Experience: 0 to 5 years) and group II (Experience 6 to 10 years) was higher than group IV (Experience: 16 to 20 years), III (Experience: 11 to 15 years) and V (Experience: 21 years and above). Similarly the coiled tail count was significantly higher in IV (9.58 ± 1.90) followed by group I (8.11 ± 1.46), II (6.13 ± 1.91), III (4.30 ± 1.30) and V (4.27 ± 0.90).

The profile of various groups of AI technicians (age, experience, trainings, location and level of education) revealed non-significant difference. The corresponding overall mean post-thaw motility and progressive motility percentage of the above profile parameters were 73.59 ± 13.57 and 50.67 ± 1.52 ; 73.58 ± 13.57 and 50.67 ± 11.99 ; 73.59 ± 13.57 and 50.67 ± 11.99 ; 73.58 ± 13.57 and 50.67 ± 11.94 ; 73.59 ± 13.57 and 50.67 ± 11.99 , respectively.

Motile mean elongation, slow concentration, slow count, slow sample, total concentration, total count and total sample were found significantly higher from the

samples collected from AITs who received basic training. The corresponding values for these parameters were 0.55 ± 0.01 , 15.08 ± 1.01 , 15.08 ± 1.01 , 171.31 ± 10.40 , 3.31 ± 0.24 , 80.31 ± 4.39 and 923.62 ± 55.66 , respectively.

5.3 Reproductive Performance of Buffaloes

Age at first AI (months) in buffaloes of Sabarkantha district was lower 35.38 ± 1.13 than the age at first AI for Arvalli and Panchmahal districts. The corresponding values were 36.50 ± 1.42 and 37.95 ± 4.97 , respectively. Similarly the age at first calving (AFC) in buffaloes of Sabarkantha district was lower than the buffaloes of Arvalli and Panchmahal districts. The AFC of buffaloes was 46.29 ± 2.26 , 55.08 ± 3.92 and 55.08 ± 3.92 months for Sabarkantha, Arvalli and Panchmahal district, respectively.

The open period in buffaloes of Sabarkantha district was 125.32 ± 2.97 days, and this period was similar for Arvalli and Panchmahal districts (156.63 ± 22.4); while calving interval in buffaloes of Panchmahal district was 442.89 ± 13.75 days followed by 440.67 ± 4.11 and 423.29 ± 8.97 days for buffaloes of Arvalli and Sabarkantha districts, respectively.

Conclusions

The following conclusions are drawn from the present study:

1. AI technicians' profile parameters had an influence on the success rate of AI with majority of the AI technicians being educated with higher educational level.
2. Refresher AI trainings at regular interval had impact on adoption of skills and knowledge of AI technicians which was reflected in significantly higher conception rate.

3. Eighty per cent of the AI technicians have performed timely insemination, whereas 53% of the AI technicians have used standard thawing temperature and time.
4. Post - thaw and progressive motility showed non-significant differences and were higher in districts like Sabarkantha followed by Arvalli and Panchmahal.
5. The overall mean values of velocity of post-thawed spermatozoa showed non-significant differences in relation to age groups of AI technicians.
6. Higher linearity (LIN) and linear motility in the age group IV (Age: 51 years and above) can be attributed to the better handling of semen straws by the AI technicians of this group.
7. Significantly higher coiled tail count and concentration were observed in experience group IV (16 to 20 years) of the AI technicians indicating failure to handle the frozen semen as per Standard Operating Procedure (SOP).

There is a dire need to take up further studies in such buffaloes to correlate CASA traits of fresh and frozen thawed semen with sperm motility, live sperm percentage and reproductive performance of the buffaloes.

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