Bhutan Journal of Animal Science (BJAS)
Volume 6, Issue 1, Page 1-11, March 2022

EFFECTIVENESS OF ASSISTED REPRODUCTIVE TECHNOLOGIES FOR NATIONAL DAIRY HERD IMPROVEMENT IN BHUTAN

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ABSTRACT: Assisted Reproductive Technologies (ARTs) have been the game changer in transforming dairy herd composition and enhancing milk production. For better understanding the efficiency and effectiveness of applied ARTs, data on semen cryopreservation and Artificial Insemination (AI) performed for the past many years, estrous synchronization protocols applied in cows/heifers, 25 trials on embryo flushing/ Multiple Ovulation and Embryo Transfer (MOET) conducted and field application of sexed semen technology were validated and analyzed. Findings suggest that AI coverage in the country is low (12%). However, over 87% of frozen semen available for AI services is produced within the country. In-country semen (pedegree selected) and imported conventional semen (progeny tested) gave birth to equal percentage of crossbred progenies in the field hinting that semen produced within the country meets required standards. Strengthening of in-country progeny testing scheme to select superior sires and gearing towards genomic sire selection will further improve semen quality. Estrous synchronization with CIDR-B/TRIU-B resulted in higher response rate than simple ovsynch protocol ($p<0.008$). Nevertheless, synchronization and fixed timed AI is ineffective when body condition score is ≤2.4 because such animal when inseminated rarely get pregnant. Hence timing syncronization during lush season when body condition of animals is optimum (2.5-4.5) can result in higher pregnancy rate. Introduction of MOET trial at the research station has recovered 19.6% (31) viable embryos for cryopreservation. However, embryo flushing/MOET being complex and resources intensive procedures, its scope for wide-scale application in the field is very limited at the moment. Advancement of research on Ovum Pick-Up and In Vitro Fertilization is necessary to improve efficiency of MOET program. Sexed semen technology resulted in 89.6% female birth. But for effective utilization of costly sexed semen, its application is recommended preferentially in virgin heifers for higher conception rate. The current study concludes that among sets of ARTs tested and applied, AI will continue to be the most viable option for cattle breed improvement program in Bhutan. Hence, reinforcing AI services combined with other ARTs that are efficient, feasible and viable can bring about substantial socio-economic benefits to the farmers.

Keywords: Artificial insemination; assisted reproductive technologies; estrous synchronization; multiple ovulations; sexed semen.

1. INTRODUCTION

Assisted Reproductive Technologies (ARTs) is defined as "any technique that interferes with the normal biological pathways of reproductive-related events and/or structures in order to produce a healthy offspring" (Velazquez 2008). Semen cryopreservation and Artificial Insemination (AI) is the first ART applied to cattle world-wide. The technology was initially tried in Russia and Denmark during the early1900s (Ivanoff 1922; Perry 1945). The next major development involved was discovery of sperm cryopreservation with glycerol in 1949 (Moore & Hasler 2017) that enabled use of frozen bovine semen. The primary thrust behind AI is widespread use of sires with genetic merit to increase the rate of genetic gain in livestock populations. Approximately, 130 million cattle are inseminated artificially annually across the globe and AI continued to be popular because its application is simple, economical and successful (Vishwanath 2003). The National

Artificial Insemination Program (NAIP) in cattle started in Bhutan in 1987. Decades of efforts on semen processing and cryopreservation, and implementation of NAIP resulted in marked shift in composition of dairy herd in favor of crossbred cattle, with positive outcome on enhanced milk production in the country (Tshering & Tamang 2018). Crossbred cow is reported to produce 2.4 to 4.6 times higher milk when compared to local cow (Samdup 2018) and contributes a major share (>59%) of milk produced at the village farms (Tamang & Dorji 2017). Estrus synchronization or estrous induction with hormones for Fixed Time Artificial Insemination (FTAI) is alternate ART applied in dairy breed improvement program. Further to trap genetic potential of superior female and elite males combined, Multiple Ovulation and Embryo Transfer (MOET) are tried in many countries. MOET in cattle were developed in the 1940s and 1950s (Casida et al. 1943; Rowson 1951; Dziuk et al. 1958). Large-scale MOET operations started in North America in 1970s, in Europe in 1980s, and in South America by 1990s (Hasler 2014). For production of offspring of pre-determined sex, sexed semen usage is field-tested in Bhutan since 2014. Flow cytometry, the proven technique for sexed semen production separating X and Y chromosome was validated by researchers of United States, Department of Agriculture at Livermore, California, and Beltsville, Maryland (Johnson & Welch 1999). The technology was patented as "Beltsville Sperm Sexing Technology" and commercialization of sexed semen started in United States in 2001 with a license granted to Sexing Technologies, Texas (National Dairy Development Board [NDDB] 2015). The Ovum Pick-Up **(**OPU**)** for repeated oocyte recovery from elite female donors was developed during the late 1980s (Pieterse et al. 1988) and In-Vitro Fertilization (IVF) for embryo production was developed in the 1990s and combining OPU/IVF with MOET is reported to improve efficiency of the MOET Program (Looney et al. 1994). Despite applications of ART in the country in an accelerated mode, commercialization of dairy farming gaining its popularity, many farmers/entrepreneurs venturing to scale-up dairy farms is facing shortage of dairy heifers. Import and supply of dairy cattle to farmers as interim measures were taken-up since 2010 (National Dairy Research and Development Centre [NDRDC] 2018) and had experienced the incursion of exotic diseases into the country and poor adaptability of imported animals in new farming environment. The sourcing and supply of dairy cattle was found to be risky and unsustainable. Hence, application of evolving ARTs such as sexed semen to meet increasing demand of heifers is then pursued as one of the viable options in Bhutan. Nevertheless, the benefits of using ARTs such as use of sexed semen, estrus synchronization and MOET vary depending upon the production system; the potential benefits have to be weighed against the cost before specific recommendations can be made regarding their wider use (FAO 2011).

Since efficiency and effectiveness of ARTs applied in Bhutan is hardly assessed, its usefulness remained less understood and undocumented. Thus, to have deeper understanding on effectiveness of ARTs applied; there was a critical need to assess and analyze the available information so as to devise appropriate recommendations for improvement of dairy cattle breeding program in the country. Hence, this study was undertaken to assess effectiveness of different ARTs applied to improve dairy breeding program and understand the prospects and challenges on effective application of ARTs in dairy cattle

2. MATERIALS AND METHODS

2.1 Data collection

The cattle population parameters for 2019 (Department of Livestock [DoL] 2020) were compiled and analyzed to uncover herd dynamics, breedable female population and AI coverage in the country. Data collected and maintained on semen cryopreservation and Liquid Nitrogen (LN_2) produced at NDRDC for last five years, field data on AI performed and progeny born for thirteen years (July 2007 to 2020) were validated. Data of field trials on Estrous Synchronization and Fixed Time Artificial Insemination (FTAI) in 95 cows/heifers that fulfill the required protocol were screened. Under this trial protocol, the animals were treated with Gonadotropin Releasing Hormone (GnRH) and Prostaglandin (PGF2α) - simple ovsynch (0-7-9 day) protocol (n=44), whereby animals received 5ml i.e., 20 µg Buserelin

Table 1: Embryo Flushing Protocol/Standard Operating Procedures

Days	Procedures	Remarks
Day 0	Insert CIDR-B (Estrous Synchronization)	Inhibits FSH & LH to introduce new wave
Day 4	6AM: Inj. FSH (1.8 ml), 6PM: Inj. FSH (1.8 ml)	Promotes multiple follicle development
Day 5	6 AM: Inj. FSH (1.4 ml), 6 PM: Inj. FSH (1.4 ml)	-do-
Day 6	6 AM: Inj. FSH (1.0 ml) +Inj. PGF2 alpha (2.0 ml) 6 PM: Inj. FSH (1.0 ml) +Inj PGF2 alpha (1.0 ml)	Corpus Luteum (CL) regression resulting in estrous after 2 – 5 days
Day 7	6 AM: Inj. FSH (0.6 ml) + Remove CIDR-B 6 PM: Inj. FSH (0.6 ml) +1st AI (if animal is in standing heat)	Promotes multiple follicle development
Day 8	7 AM: 2nd AI, 1 PM: 3rd AI (depending of standing heat)	Multiple AI to ensure optimum fertilization
Day15	Embryo Flushing	

acetate, a GnRH analogue via Intra Muscular (I/M) injection on day 0 (zero), Lutalyse - a naturally occurring prostaglandin 5ml *i.e.*, 25mg injected on day 7 I/M and 20μg GnRH on day 9 followed by AI after 24 hours (Ghuman et al. 2014; Hasler et al. 2017). This protocol was compared with animal s treated (n=51) with Controlled Internal Drug Release-Bovine (CIDR-B) or its analogue TRIU-B which are intra-vaginal device containing progesterone + PGF2α protocol. CIDR-B/TRIU-B was inserted on day 0 (zero), removal was on day 7 and injection of 5ml i.e., 25mg PGF2α I/M on the day of removal, followed by AI after 48 hours (Keslar 2002). Besides, data generated from 25 embryo flushing trials conducted from 2014 to 2019 on 77 donor cows (68 *Thrabam* & nine Jersey cross cows) were assimilated for analysis. For embryo flushing, 192mg or 9.6ml of Follicle Stimulating Hormone (FSH) per donor cow with a descending dose level was injected twice daily (6AM/6 PM) for four consecutive days (4^{th} to 7^{th} day) and 3ml (15mg) $PGF_2\alpha$ injected on the second last day i.e, on 6^{th} day (AM & PM injections) followed by AI and embryo recovery as per the Embryo Flushing Protocol/Standard Operating Procedures is presented in (Table 1).

2.2 Data analysis

Statistical Package (Minitab version-18) was used to analyze quantitative data. Data collected from the systematic reviews and primary studies were tabulated. ANOVA and t-test was applied for comparative analysis of variations in treatments to determine level of significance at 95% confidence level. Descriptive statistics: mean, standard deviation, Standard Error and percentage were also used to analyze quantitative variables. Qualitative data pertaining to prospects and challenges of ARTs application were synthesized from the information received from key informants.

3. RESULTS AND DISCUSSION

3.1 National dairy herd dynamics

The estimated proportion of adult females in the national herd is 60% of total cattle population. Johnson (1998) reported similar cattle herd composition with a maximum breeding female in the herd in Senegal, Africa. This indicates that males are likely to be disposed of with preferences of owners to keep female animals in the herd as long as they are able to breed and produce calves. Among improved crossbred cattle, heifer constitutes 35% of the population. Hence, the use of sexed semen recommended for heifers will be possible in this population group. Further, out of 97,655 AI performed using conventional semen (2007-2020), 15,823 (45%) were male and 19,573 (55%) were females progenies inducted in the dairy herd. As farmers require replacement heifer in their herd, the ratio is favourable even when conventional semen is used.

3.2 National AI Coverage

Based on estimated breedable female population (both crossbred and local cattle) and mean AI done per year, AI coverage at the national level is estimated to be 12 % (Table 2). The findings that are inaccessible to AI facilities or inadequate awareness of farmers on AI services. Providing adequate advocacies to farmers on the benefits of AI and encouraging mobile AI services can

Table 2: AI coverage in different regions and at national level

Regions of the country	Female cattle population	Breedable female	Mean AI done/year	AI Coverage (%)
East Central region	22008	12104	2244	18.5
Western region	37757	20766	1776	8.6
West Central region	25743	14159	1427	10.1
Eastern region	37366	20551	2637	12.8
National Level (total)	**122874**	**67580**	**8084**	**12.0**

Note: AI Coverage=Breedable female divided by AI done/year (mean AI done for five years). Total female population include exotic & local cattle other than Mithun cross, breedable female is estimated at 55% of total female cattle population as not all adult females are breedable. Data source: Livestock Statistics, 2019 (DoL 2020)

increase AI coverage. Similar findings were reported with the national level AI service of less than 15% of the breedable cattle population in Sri Lanka (Abeygunawardena and Alexander 2001).

These findings are lower than world average of 20% (Thibier & Wagner 2002). Thus, there is a need to address the current challenges in cattle breeding program and upscale AI coverage through different interventions to catch-up with world average.

3.1 Adoption of ART to intensify Dairy Breed Improvement Program

3.1.1 Frozen semen processing and cryopreservation

Frozen semen processing is one of the most important ARTs that has played very crucial role in dairy breed improvement program in Bhutan. With a mean production of 24,967 doses of frozen semen per annum, worth Nu. 2.9 million (based on calculated cost of production of Nu. 117/dose); it contributes to curtail semen import substantially. Locally produced frozen semen constitute 87% (1,50,287 doses) of stock in the semen bank for further distribution (NDRDC 2020). Chupin & Schuh (1992) supplemented the view that locally produced semen represents more than 50% of the semen available in 40 semen-producing countries and has huge impact in cattle breeding. To preserve the frozen semen, on an average 369 liters of LN_2 is required per year for each Artificial Insemination Centre (AIC). Further major chunk of LN_2, about 38% (15,646 liters) of mean 41,005 produced annually is used to preserve frozen semen at the national semen bank maintained at NDRDC and saves Nu. 1.55 million annually (based on calculated cost of production of Nu. 38/ litre).

3.1.2 Artificial Insemination

Thirteen years data on AI performance (July 2007 to June 2020) shows that a total of 97,655 AI was performed and 35,396 progenies were born with mean success rate of 36.2% at the national level. Analysis of Dzongkhag-wise and Region-wise data on total AI performed and total progeny born (July 2013- June 2020) revealed that the Eastern region has performed the highest number of AI with Mongar Dzongkhag (District) topping the list. It is followed by western region (Paro dzongkhag) while Gasa followed by Haa dzongkhags performed least number of AI (Figure 2). This could be attributed due to unfavorable farming environment (high altitude, yak priority area) with sparse breedable cattle population.

The above finding is lower than the success rate of 39.6 % for conventional semen used in commercial farms in Australia (Healy et al. 2013). Similarly, Nebel (2002) reported that during 50 years of AI practiced in the United States, the fertility of virgin heifers has remained relatively constant at approximately 65% in first service conception; whereas, conception rate for lactating cows is about 40%. Further, loss of pregnancy due to embryonic death is seldom observed by Bhutanese farmers. But it is reported that success rate even in well managed herds is undermined by losses of pregnancy characterized by early embryonic death which occurs prior to the period of Corpus Luteum (CL) maintenance in the cow at days 15-17 of the

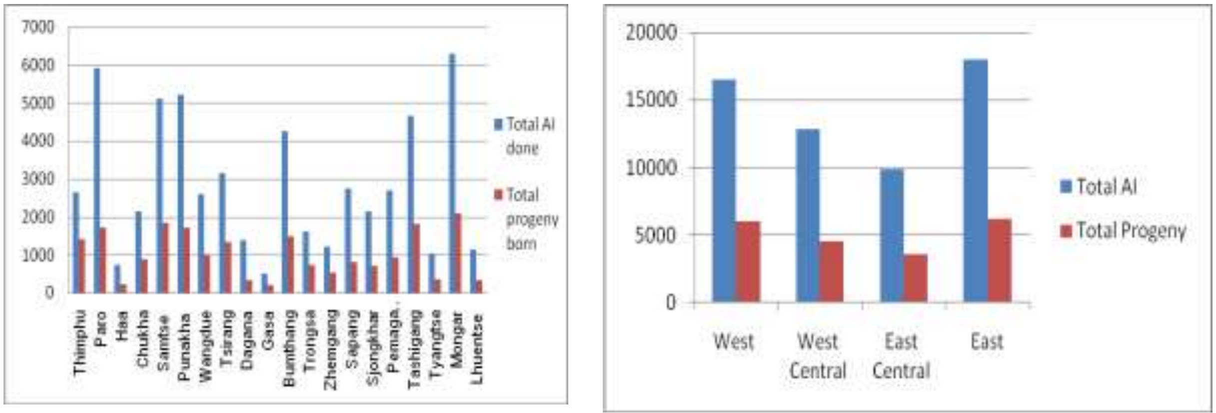

Figure 2: Dzongkhag-wise and Region-wise total AI done and progeny born (2013-2020)

Table 3: Progeny born from progeny tested imported semen and pedigree selected local semen

Breed of cattle	AI with imported semen (CHBPP)	Progeny born (CHBPP)	Progeny born %	AI done (local semen) (non-CHBPP	Progeny born (local semen)	Progeny Born %
Jersey	16538	6580	39.8	33416	11350	34.0
Brown Swiss	737	265	35.9	609	241	39.6
Total /Mean	**17275**	**6845**	**37.8 (mean)**	**34025**	**11591**	**36.8(mean)**

cycle, and late embryonic death, which occurs from CL maintenance to the end of the stage, at approximately 42 days of gestation (Santos et al. 2004).

Embryonic mortality is estimated to range from 10 to 40% in first-service heifers to 65% in repeat-breeder cows (Goeseels & Kastelic 2003). Chromosomal abnormalities, poorly developed embryos, inadequate uterine environment and infectious agents are reported to be some of the causes. Measures to minimize causative agents; technologies such as ultrasonography and Pregnancy Associated Glycoprotein tests to improve accuracy of early pregnancy diagnosis needs to be popularized for timely corrective measures to improve reproductive efficiency in a dairy herd.

3.1.3 Use of imported progeny tested and pedigree selected semen

The selected villages and the communities participating in Contract Heifer & Bull Production Program (CHBPP) in the country is supplied with progeny tested (PT) imported frozen semen while the non-CHBPP area are supplied with pedigree selected semen produced within the country (Table 3). Table 3 above shows that progeny tested imported semen and in-country produced pedigree selected semen is comparable with progeny born percentage of 37.8% and 36.8% respectively. This indicates that semen produced in-country meets required standards. Progeny tested imported semen however is reported to be slightly better than pedigree selected semen because PT sires are intensively selected. Thus, there is a need to strengthen in-country progeny testing scheme to select superior sires and build capacity to progress towards genomic selection of sires.

3.1.4 Estrous Synchronization and Fixed Time Artificial Insemination

Estrous synchronization and FTAI data a simple ovsynch protocol (n=44) and Intra Vaginal Device (CIDR-B/TRIU-B) protocol (n=51) revealed that latter gave significantly higher response rate to follow-up with FTAI ($p<0.008$), suggesting that this protocol is better option for inducing estrous in cattle (Table 4). Similar studies on estrus synchronization using CIDR-B and GnRH-PGF2α based ovsynch protocol suggested that former was better to improve reproductive efficiency of non-

Table 4: Response rate with Intra Vaginal Device and Simple ovsynch protocol

Synchronization Protocol	N	Animal Response (%)	Response rate (mean)	± SE	*p*-value
Intra Vaginal Device (CIDR-B/TRIU-B)	51	93	18.96	3.47	0.008
Simple ovsynch (GnRH-PGF2α)	44	71	4.79	1.09	

descript cows during the low and peak breeding seasons (Haider et al. 2017) and it was found to be superior to ovsynch for synchronization of ovulation and subsequent conception rate in buffalo (Ghuman et al. 2014). Lesser response to GnRH-PGF2α in the current study could be because the stage of follicular growth may not have been assessed at the time of initial GnRH injection owing to inadequate skills of field staff while advocating this protocol. However, Tamang et al. (2020) observed that even if there is good response in animals that were estrous synchronized, FTAI performed in such animals have not readily translated into pregnancy and calf birth when Body Condition Score (BCS) is ≤ 2.4 (on a scale of 1 to 5).

The calving rate further worsened when local cows/heifer were synchronized and inseminated during winter months, with success rate of only 14%. Similar research findings from South Africa and Ethiopia indicated that the estrus response differed among cattle breeds, varied across the agro-ecologies with low pregnancy rate in cows/heifers that had BCS of <2.5 (on a scale of 1 to 5). However, it improved when BCS was 3 to 4.5 (Maqhasu et al. 2016; Legesse 2016). Thus, for better results application of synchronization protocols, selection of animals with optimum BCS is prerequisite.

3.1.5 Application of MOET Technology

The response rate to 192 mg/9.6 ml (20mg/ml) of FSH treatment for superovulation of donor cows is found satisfactory. Most donor cows irrespective of breed responded well to the treatment which was confirmed through palpation of the ovaries at the time of flushing. In 25 embryo flushing trials performed as per the standard procedures, 158 embryos were recovered of which 31 (19.6%) viable embryos (morulla stage) are cryopreserved in semen/embryo bank at NDRDC. Details of embryo recovery are presented in (Table 5). When embryo flushing was conducted at National *Nublang* Breeding Centre (NNBC), mean number of embryos recovered from local *Thrabam* cow was 8.3 and total number of viable embryos cryopreserved were 23 which is higher than mean 1.9 embryo recovered from same breed of cattle at NDRDC, Yusipang. This could be probably because the animals selected at NNBC had better BCS and with flushing done during May-June could have coincided with optimal season. Hence, favourable season/weather conditions and selecting donor cows with good BCS is likely to result in better embryo viability/viable embryo recovery.

Embryos recovered from Jersey cross (JX) cows were significantly higher than *Thrabam* cows (p<0.016). Mean number of embryos recovered from JX was 17.3 with four viable embryos when compared to *Thrabam* cow (mean 8.30). This suggests that embryo recovery is better in JX cattle probably because of higher fertility rate due to infusion of exotic dairy breed inheritance. However, donor variation is observed within the breed with some donors consistently producing

Table 5: Embryo flushing trials carried out & viable embryos cryopreserved

Trial location	Nos. of embryo flushing trials	Nos. of Donor cows	Breed of cattle	Total embryos recovered	Mean embryos recovered per flushing	Viable embryos recovered (Nos)
NDRDC	12	36	*Thrabam*	23	1.90	4 (17.4)
NDRDC	3	9	JX	52	17.30	4 (7.6)
NNBC	10	32	*Thrabam*	83	8.30	23(43.4)
Total	**25**	**77**		**158**		**31(19.6)**

Note: *figures in parenthesis are percentage, JX means Jersey cross, Thrabam (female) & Nublang (male) is term used for local cattle breed* more viable embryos than others and also varied between different embryo flushing which appear to be uncontrollable and unpredictable. Further, ET procedures being complex and very structured program, high level of precision and skills of professionals and their time accuracy is critical to obtain the best results.

There are variations in the success of MOET and application of the technology in advance countries. Commercial embryo transfer (ET) in North America is reported to be static or declining whereas in South America it is expanding, accounting for 22% of ETs throughout the world (FAO 2007). Alarcon and Galina (2009) reported that government organizations in Mexico have initiated programs to popularize MOET, particularly in small-scale enterprises (<50 cows unit). However, based on their analysis, ET is not a viable technology for the farmers to sustain on their own owing to cost involved in the preparation of the donor and recipient cows, embryo recovery and the resulting gestation. Hence, profitability, acceptance and upscaling of MOET technology for small scale farms in the developing countries including Bhutan is still a challenge.

3.1.6 Sexed Semen Technology application

Sexed semen technology pre-determines sex of calf in favor of female progeny. In Bhutan and so is in some other parts of the world, female dairy calves are generally reared as replacement heifers. Male calves especially with exotic inheritance such as Jersey/Jersey crossbreds are unwanted in a herd because of its unsuitability for draft power and religious sentiments associated with culling for meat purpose. Production of surplus male offspring thus harnesses a socio-ethical concern in the dairy industry. Balzani (2020) studied the Irish dairy sector on problems of male dairy calves in dairy industry and recommended that use of sexed semen can be one of the mitigation strategies to reduce the production of surplus male calves.

In order to reduce burden of male calves and produce higher number of female calves needed by farmers, application of sexed semen technology was tried in Bhutan since 2014 and the technology was officially released in July 2020 for its wider application (NDRDC 2021). Initial trial results documented a female birth of 89.6% with mean conception rate of 44% (Rai et al. 2019). The finding on female birth is higher than 86% female calves birth reported in commercial farms in New South Wales, Australia using sexed semen (Healy et al. 2013). However, conception rate is similar to result of DeJarnette and coworkers (2009), who reported that heifer pregnancy rates of 45% and 56% for sexed and conventional semen respectively.

Owing to lower sperm concentration in sexed semen, lowered fertility due to damage to sperm during sorting there is 10% to 20% decrease in conception rates with sexed semen compared to conventional semen (De Jarnette et al. 2009; Balzani et al. 2020) and its use in estrous synchronization and FTAI has been discouraged (Seidel 2003). Moreover, because of its higher cost per dose of semen, combined with a reduced conception, sexed semen is recommended preferentially in virgin heifers (De Vries 2019). Nevertheless, using sexed semen alongside genomic testing increased genetic gain, fetched higher sale price by heifer compared with a male to make sex semen use profitable provided conception rates is not lower than 10% compared to conventional semen (Newton et al. 2018).

To weigh benefits of sexed semen over its cost, cost of production of calves at birth (both male + female) and female alone was estimated. Estimation was based on assumption that a minimum of 72 AI (average 6 AI per month) is performed annually per AI Centre in Bhutan, applying mixed breeding strategy (36 AI with sexed semen and 36 AI with conventional imported or pedigree semen). Other costs remaining constant, owing to high cost of sexed semen (US$14 or Nu 1073.1 per dose) compared to US$ 3/dose (Nu 229.95) for imported conventional semen, and Nu 117 for pedigree semen, when sexed semen is used, variable cost was higher by 51% compared to imported conventional semen and 62% higher than pedigree semen. However higher number of female births with sexed semen; cost per female calf born from sexed semen is only 4% and 11% higher than imported and pedigree semen respectively (Table 6). The calculated cost of production of heifer (18 months old) at the Bhutanese village farms is

approximately Nu.38,175 (NDRDC 2021) which excludes cost involved till birth of a calf. Research elsewhere also indicated that with all factors kept constant, AI with continuous use of sexed semen to break-even the net present value i.e., to equal continuous conventional semen use, the newborn female calf must be worth at least $226.13(Nu. 17,332) and heifer must fetch a selling price of at least $512.81 or Nu.39,307 (Olynk & Wolf 2007).
In more recent finding, making use of sexed semen is reported to be profitable when the value of the heifer calf is at least $400 (Nu.30,660) more than the value of the bull calf (valued at US$ 110, Nu. 8431.5) but not profitable in dairy cows, unless the fertility is almost equal to conventional semen (De Vries 2019). Thus, the economic gains from use of sexed semen are likely to depend on number of female calves born that enter adulthood with minimal mortality and selling price of heifers.

3.2 Prospects of ART applications in dairy

- In-house semen cryopreservation capacity has a positive effect in genetic up-gradation of cattle population in the country and improved capability can yield positive results.

Table 6: Comparison of cost of production of AI calf using different semen types

Parameters	Cost of sexe semen /deprecia on fixed asse	Cost of conventional semen/depreciation on fixed asset	Cost of pedigree semen depreciation on fixed asset
Fixed cost			
Depreciation AI crate/yr (@ Nu. 30,000)	1250	1250	1250
Depreciation o LN2 cans/yr (@ Nu.60336)	5534	5534	5534
Depreciation AI gun/ yr (@ Nu1650)	140	140	140
Total fixed cost	**6,924**	**6,924**	**6,924**
Variable cost			
Semen cost /yr (36 AI per semen type)	38631.6	8278	14212
Cost of LN2(1L/AI /day)	1368	1368	1368
AI Service delivery cost (50% DSA @ Nu.625)	22500	22500	22500
AI accessories sheath/ gloves/yr	162	162	162
Transport cost to reach LN2 from LN2 plant to AI Centre @Nu13/L	4797	4797	4797
Travel cost: reaching LN2 to field AI Centre	22500	22500	22500
Total variable cost	**89,958**	**59,605**	**55,539**
Total fixed +variable costs	**96,882**	**66,529**	**62,462**
Total progeny born (30% CR sexed & 37% other semen)	11	13	13
Cost of calf at birth (male + female**)**	**8971**	**4995**	**4689**
Mortality (5%)	1	1	1
Net number of calves survived	10	12	12
Female calf born	9	7	7
Cost of female calf born	**10,492**	**10,080**	**9,464**

Note: *1US$=76.65 (7 dec 2021); Cost of LN2: 1L/AIC/day (369L LN2 required /AIC/yr), CoP Nu.38/L LN2; LN2 transportation from Yusipang /Kanglung to Dzongkhas/Geog estimated to cost Nu 13/L (400km/trip x 9 trips (every 42-45 day) i.e., fuel +maintenance of vehicle; conception rate 30% sexed, 37% other semen): 90% female birth for sexed and 55% conventional semen, mortality= 5% of progeny born, DSA means Daily Sustenance Allowance. Depreciation cost calculated using formula: Actual value(minus) Salvage value(estimated) divided by useful life.*

- AI service can be intensified using progeny tested sexed semen in heifer and conventional semen to cover rest of the female cattle population (mixed-breeding strategy) in all feasible areas to create a visible impact.
- Regular refresher course provided to field staff can help to refine techniques of artificial insemination; developing skills of inseminators to manoeuvre inseminations which shall enable to adopt best practice to maximize the success rate.
- Wider application of sexed semen technology can increase availability of replacement heifers to scale-up dairy farming to commercial mode; ensures farm biosecurity thereby avoiding introduction of disease through import of cattle
- Research and assessment are in progress to identify ART that are efficient and cost effective, before its wide scale application and its benefits to dairy farming communities in a long-run

3.3 Challenges of ART applications in dairy

- Rugged terrain with limited road access is a hindrance for rapid uptake of AI Technology. Mountain environment with far flung villages away from nearest AI centre make AI service delivery inaccessible
- Mobile AI service is possible to some extent but limited mobility facility is a bottleneck
- Limited availability of economically priced Liquid Nitrogen for semen cryopreservation and high cost of imported sexed semen is impeding intensification of AI services
- MoET research requires considerable skills, experience and resource, hence technology transfer to field is still a long way to go

4. CONCLUSIONS

Currently the most cost-effective way to disseminate genetic merit is undoubtedly with conventional Artificial Insemination services. However, AI coverage in Bhutan only about 12% of breedable females population, thus, a concerted effort is required for intensification of this technology. In-country produced pedgree semen has almost the same progeny output as that of imported conventional semen indicating that required semen production standard is met. But for further improvement in quality of semen produced within the country, on-going progeny testing scheme for selection of superior sires needs to be strengthened and capacity needs to be built to undertake genomic sire selection in a longer run. Estrous synchronization and FTAI with CIDR-B/TRIU-B is found to be a better option than other protocols and it is suggested to continue as part of reproductive waste management program in the field. However, estrous synchronization and FTAI is unlikely to result in pregnancy when animals in sub-optimal body condition is <2.5 (0-5 scale). Thus, choice of proven dairy breed such as Jersey and Holstein Friesian with higher fertility rate than local cattle, selection of animals with optimum BCS, and applying synchronization techniques during lush season when there is sufficient fodder for animals to remain healthy can bring about better outcome. MOET is being tried with viable embryos recovered and cryopreserved. Lesson learnt in improving embryo recovery includes: right season of flushing, selection of healthy donors of suitable dairy breeds with exotic inheritance and enhancing competency of flushing personnel. Nevertheless, owing to high cost involved in the preparation of the donor and recipient cows, embryo recovery and successful transfer, MOET program is foreseen to be costly for farmers to sustain such program on their own without substantial incentive support from Government. Advancement of research on OPU/ IVF programs can improve efficiency of MOET programs that needs to be introduced in Bhutan in the near future. Sexed semen technology application is promising and its usage has to be expanded in all feasible areas to help farmer, groups and cooperatives in producing a greater number of replacement heifers. However, high cost per dose of sexed semen and reduced conception rate, implementation of mixed breeding strategy is suggested whereby sexed semen is used exclusively in virgin heifer because of its higher fertility than cows while conventional semen is used in multipara cows. In general, improving AI coverage, availability of dairy inputs especially LN_2 and quality frozen semen for higher success rate, improvising AI techniques through up-skilling/re-skilling of Inseminators and strengthening on the application of viable ART that are efficient and feasible will have a noticeable result and a positive growth under dairy sector.

Acknowledgements

Volumes of data for several years on AI and progeny record, breedable females, field trials records on estrous synchronization and FTAI provided by Mr. Abi Narayan Koirala, Sr. Livestock Officer, NDRDC; five years data on frozen semen and LN_2 production and distribution by Mr. Phub Dorji, LN_2 Plant Manager, NDRDC, is the major inputs for this paper. Authors gratefully acknowledge the support from both **of** them.

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