

# **Reproduction Efficiency in Dairy Bovine:** Trends and Targets

## A. Kumaresan and A. K. Srivastava

#### Abstract

Reproductive efficiency is the major factor determining productivity in dairy bovine. In spite of several technological advancements, poor fertility of dairy bovine continues to be a major factor limiting the profitability of dairying. Fertility is a multifactorial trait influenced by genetics, environment and management, and their complex interactions, making it difficult to determine the precise reason for poor fertility. Over the period, the dairy bovines have been selected for high performance; milk productivity has steadily increased due to genetic improvement combined with better nutrition and management. On the other hand, selection of animals for high milk production has also changed the metabolic adaptation and reproductive physiology of animals, leading to decreased reproductive efficiency. Several researchers reported that greater milk production was associated with reduced reproductive performance in dairy cattle. However, an interesting fact is that declines in fertility are observed in the lactating cows rather than for the heifers, indicating that the impact of genetic selection for higher milk production on inherent fertility is likely to be modest. This offers scope for application of modern reproductive technologies coupled with environmental, managemental, and nutritional interventions to improve the reproductive efficiency in the dairy bovine. In this chapter, targets for dairy bovine reproductive efficiency, how far the set targets are achieved, the possible reasons for

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A. Kumaresan, A. K. Srivastava (eds.), *Current Concepts in Bovine Reproduction*, https://doi.org/10.1007/978-981-19-0116-4\_2

reduced fertility, and an overview of reproductive technologies for improving fertility are discussed.

#### **Keywords**

Dairy bovine · Reproductive efficiency · Targets · Reproductive technologies

#### 2.1 Introduction

Dairy bovine productivity largely depends upon reproduction, which in turn is influenced by several factors including genetic, nutritional, hormonal, physiopathological, and management practices (Pryce et al. 2004; Veerkamp and Beerda 2007; Löf et al. 2007). Reproductive disorders and infertility in dairy bovine pose serious economic loss to farmers by reducing returns and increasing veterinary expenses. The target for successful reproduction efficiency in dairy cows is to accomplish "a calf per cow per year" and in case of buffaloes, it is "a calf per buffalo cow in every 14–15 months." To achieve this target, a cow or buffalo should conceive within 85–115 days postpartum, which in turn requires that the animal should start cyclicity as early as 45 days and breeding should start by 60 days postpartum. But this is seldom achieved because of postpartum complications including prolonged acyclicity, uterine infection, and negative energy balance (Dobson et al. 2008; Lucy 2008; Sheldon et al. 2008).

Several studies have reported a decreasing trend in the fertility of dairy cows over a period of time. Trends of greater numbers of days open, services per conception, and extended calving intervals have been reported in dairy cows over the past 25-35 years. A report by the Swedish Dairy Association indicated that the calving interval of Swedish Holstein cattle increased to 13.6 months in 2012 from 12.5 months in 1985 (Swedish Dairy Association 2012). In general, high production levels selected for dairy herds have been reported as a reason for fertility decline in dairy cattle (Hansen 2000; LeBlanc 2010); however, a clear consensus it yet to be arrived. While several studies have shown clear negative genetic relationship between milk yield and fertility, others have shown there are high producing dairy animals with good fertility. Although declining dairy cow fertility is a concern for several countries across the globe, some of the highest yielding and most productive herds in some countries are able to maintain outstanding fertility. This clearly demonstrates that poor fertility is not an inevitable consequence of modern dairy systems (Arnott et al. 2015). Maintaining good fertility requires a perfect matching between genotypes and the production environment, along with appropriate management practices (Rauw and Gomez-Raya 2015). Therefore, it is possible to achieve high fertility in high yielding dairy bovine through proper management, nutrition, and application of appropriate reproductive technologies.

Universally, it is well recognized that reproductive technologies have revolutionized dairy production. Recent advancements in reproductive technologies facilitated manipulation of the reproductive process in dairy animals for improving their reproductive efficiency. Reproduction technologies are considered to be good model of technology transfer between the laboratory and the farms, since the adaption of these technologies has been exemplary. In several countries, advanced reproduction technologies (e.g., ovum pick-up and in vitro embryo production) are routinely applied at grassroot level and the impact of these technologies on animal production and on the economic developments of farmers is very evident. On the other hand, in other countries, the first-generation reproduction technologies (For instance artificial insemination) are the only technologies available to the farmers and other frontier technologies are mostly restricted to laboratories. In these countries, requirement for high initial investment, infrastructure, and inadequate expertise are the few factors limiting the extensive use of these technologies. Nevertheless, the time has come up to realize the full potential of these technologies.

#### 2.2 Dairy Reproduction Benchmarks

To achieve high reproduction efficiency in bovines, a thorough knowledge on indicators of reproductive efficiency, their application in day-to-day farm operations, and overall improvement of selected reproductive parameters are important. Few important reproductive efficiency parameters are defined here and the targets for the reproductive efficiency parameters are given in Table 2.1.

- *Conception rate:* Conception rate is defined as the proportion of inseminations leading to a pregnancy. It is the number of pregnant cows over total of services in a given period of time. Although it is commonly calculated on a monthly basis, it can be used for any defined group of inseminations.
- *Pregnancy rate:* It is the result of conception rate X the service rate. It measures how fast cows get pregnant every 21 days; therefore, it is measured in a time interval of 21 days. Service rate is the number of eligible cows served within a period of 21 days.

<b>Table 2.1</b> Dairy cattle         reproductive efficiency         parameters and their targets         (Compiled from different         sources)	Parameter	Target	
	Conception rate at first artificial insemination	55-60%	
	Number of AIs required for conception	Less than 2.0	
	Calving interval	365-375 days	
	Calving to first service interval	60-65 days	
	Voluntary waiting period	45-55 days	
	Submission rate	70-80%	
	Heat detection rate	70-80%	
	Calving to conception interval	85-100 days	
	Days open	120 days	
	Nonreturn rate	>75%	
	Overall pregnancy rate	>65%	
	Culling rate due to reproductive failure	<8%	

- *Calving interval:* Calving interval represents the number of days between two successive calvings. Calving interval includes the measure of return to cyclicity and conception; however, at times, it may be biased, because infertile cows are often subjected to early culling and hence do not recalve. At the herd level, the average calving interval, across a group of lactations, is referred to as the calving index, which fairly indicates the overall picture of fertility for an all-year calving herd.
- *Calving to first service interval:* Calving to first service interval is the number of days between the day of calving and the day of her first service postcalving. This interval is influenced by herd management, which needs to be considered in fertility evaluations. While calving to first service interval is a measure of return to postpartum cyclicity, it does not provide information about conception. Excessively long calving to first service intervals indicates considerable time delays and financial losses.
- *Voluntary waiting period:* The Voluntary Waiting Period is the period after calving when cows are intentionally not allowed for mating/serving. The Voluntary Waiting Period could have an influence on first service conception rates and lactation length.
- *Submission rate:* Submission Rate is a valuable measure for all herds. Submission Rate for a month is the percentage of cows inseminated at least once during that particular month of the mating period, which begins after completion of the Voluntary Waiting Period.
- *Heat detection rate:* Heat Detection Rate is the percentage of cows correctly identified in heat out of those cows eligible for heat in a defined period. It is one of the key indicators of estrus expression intensity and estrus detection efficiency.
- *Calving to conception interval:* It is the time interval between calving and the service that resulted in pregnancy. Calving to conception interval is simply a way to measure calving interval with a shorter time lag.
- *Days open:* Days open is the period between the date of calving and the date of last insemination that resulted in conception or culling or death. For cows that conceive, days open is equivalent to calving to conception interval. For those cows not conceiving, days open is the period from calving to culling or death, or the maximum interval from calving to insemination.
- *First service pregnancy rate:* This indicates the cows/heifers conceived at the first service itself. First service pregnancy rate is the percentage of cows/heifers that conceived out of the total number of first services given over a period or to a group of animals. First service pregnancy rate is one of the most valuable measures of dairy herd fertility.
- Nonreturn rate: The nonreturn rate is the proportion of inseminated cows that are not subsequently rebred within a specified time period. NR is the most commonly used measure for bull fertility evaluations and the commonly studied periods are 28 days (NR 28), 56 days (NR 56), or 90 days (NR 90) after mating/insemination.
- *Overall pregnancy rate:* It is the percentage of animals conceived out of the total number of services given to a group of animals or over a specified period.

• *Culling rate:* The culling rate is the proportion of cows removed from the herd during a defined period (usually 12 months) out of the total number of animals.

#### 2.3 Is Fertility Really Declining in Dairy Animals?

Several studies originating from all over the globe reported a decrease in the fertility of dairy bovine. For instance, in the USA and England, it is reported that the first insemination conception rate has decreased by 0.45% and 1%, respectively, per year over a period of 20 years (Butler and Smith 1989; Royal et al. 2000a, b). On the other hand, few reports indicate some of the highest yielding and the most productive herds in the UK were able to maintain very good cow fertility. This clearly demonstrates that poor fertility performance is not an inevitable consequence in modern dairying. The reported changes in the fertility status of dairy bovine over a period of time are summarized in Table 2.2.

Both the negative relationship between milk production and reproduction, and the changes in the management practices to support high milk production, could explain the decline in dairy cattle fertility (Lucy 2007). Milk production and fertility are two economically important traits that are polygenic, affected by many genes and variants, with each gene having small effects on the observed phenotype (Snelling et al. 2013). During the past few decades, intense genetic selection has increased milk production potential of the cow, but also changed the reproductive physiology leading to a decrease in reproductive efficiency (Lucy 2001). But as on date, controversy exists among the researchers regarding the trend of dairy cattle fertility over few decades and also about the relationship between milk yield and fertility. While few report no decline in fertility, a majority reports indicate that the dairy cattle fertility is decreasing over a period of time. For instance, over the past 50 years, it has been reported that the percentage of estrus animals showing standing to be mounted activity has declined from 80% to 50% and the duration of estrus has declined from 15 h to 5 h. During the corresponding period, the first service conception rate declined from 70% to 50% (Dobson et al. 2008), while the days open and the number of services per conception has increased (Lucy 2001). Tracing back to the history of selection of dairy animals, one can evidently note that animals were selected mainly for milk production ability and reproduction parameters have not been given due importance. Based on the large datasets, a clear antagonistic relationship between milk production and reproduction has been reported in dairy cattle (Dematawewa and Berger 1998; Hansen 2000). On the other hand, few reports also indicate that the historical decrease in fertility has reached a nadir and started to improve (Crowe 2007; Norman et al. 2009); however, further studies involving large sample size are required to confirm this trend. In a review by LeBlanc (2010), it is urged to critically reanalyze the studies, which have made conclusions on the association of level of milk production with fertility based on incomplete or biased datasets. It is also indicated that improper management of high yielding dairy cows might have significantly contributed to the poor fertility rather than direct effects of genetics. A word of caution for the readers is that all these studies were conducted on

Country	Fertility parameter	Change in fertility	Reference
USA	Conception rate at first artificial insemination	Decreased by 0.45% per year	Beam and Butler (1999)
USA	Number of AIs required for conception	Increased from 1.75 to more than 3	Lucy (2001)
USA	Calving interval and conception rate	Deterioration in both measures (decrease in calving interval more pronounced)	Norman et al. (2009)
England	Conception rate at first artificial insemination	Decreased by 1% per year	Royal et al. (2000a, b)
UK	Calving interval, conception rate, and calving to first service interval	Improvement in calving interval and calving to first service interval; conception rate stable	Hanks and Kossaibati (2012)
The Netherlands	Conception rate at first artificial insemination	Decreased from 55.5% to 45.5%	Jorristma and Jorristma (2000)
Spain	Ovarian inactivity	Increased by 4.6%	Bousquet et al. (2004)
Ireland	Number of AIs required for conception	Increased from 1.54 to 1.75	Mee et al. (2004)
Ireland	Conception rate	Decreased from 64.9 to 57.1%	Mee et al. (2004)
France	Nonreturn rate	Decreased by 15%	Bousquet et al. (2004)
Canada	Nonreturn rate	Decreased from 69 to 67%	Van Doormall (2002)
Canada	Conception rate at first artificial insemination	Decreased from 44 to 39%	Bouchard and Du Tremblay (2003)
Canada	Conception rate at second artificial insemination	Decreased from 47to 41%	Bouchard and Du Tremblay (2003)
Canada	Number of AIs per lactation	Increased by 0.48 per cow per lactation	Bouchard and Du Tremblay (2003)
Canada	Submission rate and conception rate	Relatively stable	LeBlanc (2010)
Sweden	Calving interval	Increased to 13.6 months from 12.5 months	Swedish Dairy Association (2012)
Sweden	Calving interval and calving to first service interval	Deterioration in both the parameters	Löf et al. (2007)
Norway	Calving interval and calving to first service interval	Relatively stable	Refsdal (2007)
Australia	Submission rate and conception rate	Slight deterioration	Morton (2011)

**Table 2.2** Trends in dairy cow fertility reported in the literature

high yielding cows and the same may not be extrapolated to moderate or low yielding dairy animals. Based on the forgoing findings, it is logical to infer that intense selection for high milk production negatively impacted the physiology of cows leading to reduced likelihood of the pregnancy establishment; however, suitable modifications in management practices may help to improve fertility in high producing cows (Walsh et al. 2011).

Although the decline in reproductive efficiency of cows is chiefly related to changes in management, it is reasonable to attribute a portion of this decline to the males (DeJarnette et al. 2004). Several studies reported that infertility and subfertility in males costs significantly to dairy production (Larson and Miller 2000). Moreover, several scientific surveys on the dairy industry reported that fertility of bulls has also declined over a period of time (Cropp et al. 2005). Recently, the trend of subfertility in crossbred bulls belonging to three generation was studied and found an increasing trend as generation advances (Vijetha et al. 2014). The mean age at first semen collection increased by 66 days in sires compared to their grandsires. Also, the age at first semen freezing (AFSF) showed a linear increase from grand size to son. In grand sizes, the AFSF was  $767.14 \pm 25.82$  days, while the AFSF in sires was  $831.43 \pm 31.17$  days, which further increased to  $871.25 \pm 61.82$  days in son. The semen production period (SPP) was higher in grand sires compared to sires and sons (Fig. 2.1). Similarly, the sons and sires produced higher percentage of poor ejaculates compared to grand sires. Although management could be a reason for the differences in AFSC, AFSF, and SPP among grand sires, sires, and sons, the data indicates a decreasing trend in quality semen production ability. When a breeding bull suffers from subfertility/infertility, it can have a significant effect on reproductive efficiency of cows, because semen from one male is used to breed several thousand of females. In a very recent review, it is reported that low-fertile bulls had altered testicular cytology indices, reproductive hormone concentrations, sperm functional attributes, and seminal plasma composition as compared to high-fertile bulls (Kumaresan et al. 2021).

#### 2.4 How Increased Productivity Could Affect Reproductive Events?

Reduced reproduction efficiency in high yielding animals could be explained by the physiological changes associated with the increased milk production. For instance, in dairy cows, in addition to increased milk yield, greater herd size, modifications in housing conditions, and increase in do-it-yourself artificial insemination have all contributed to difficulties in achieving high fertility in high producing dairy cows (Rodriguez-Martinez et al. 2008). The possible ways by which higher milk production could be associated with impaired reproduction are indicated in Fig. 2.2. Reported physiological alterations in high milk producing cattle are indicated below.

• *Nutritional stress*: There is a rise in the energy demand in high milk-producing dairy cows. The increased energy demands could be met only partially by



Fig. 2.1 Trend in production of quality semen by crossbred breeding bulls. *AFSF* age at first freezable semen production, *ERR* ejaculate rejection rate, *SPP* semen production period

increased feed consumption (due to restrictions in feed intake and appetite). Therefore, the remainder requirements are being met by mobilization of body reserves resulting in negative energy balance (NEBAL). The NEBAL makes the animal conducive to metabolic disorders, which further leads to immune compromise condition and uncoupling of somatotropic axis. High producing cows in low body condition score (BCS) at the time of calving, or that suffer excess loss of BCS during early postpartum, are less likely to ovulate and thereby leading to an impaired reproduction.

• *Delay in resuming cyclicity*: Low BCS coupled with severe negative energy balance affects the pulsatile secretion of luteinizing hormone (LH), reduces the



responsiveness of ovaries to LH stimulation, reduces the follicle functional competence, reduces the estradiol production, and ultimately leads to abnormalities in ovulation.

- *Altered estrus expression*: Normal estrous cycles, estrus duration, and overt signs of estrus are essential for deciding the appropriate time of insemination in relation to ovulation so that conception occurs. However, high producing dairy cows have been reported to have shorter estrus (6.2 h Vs 10.9 h), reduced total standing time (21.7 s Vs 28.2 s), and lower peripheral estradiol concentrations (6.8 pg/ml) vs 8.6 pg/ml) as compared to low producing dairy cows (Lopez et al. 2004).
- *Fertilization failure*: High producing cows are highly sensitive to heat stress, which contributes to the fertilization failure in these animals.
- *Embryonic mortality:* It is reported that oocytes derived from high genetic merit cows for milk production showed inferior capacity for in vitro embryo development as compared to the oocytes derived from cows of medium-genetic merit, irrespective of the actual milk production (Snijders et al. 2000). Moreover, in lactating high producing cows, there may be asynchrony in the transport of embryo to the uterus that may result in early embryo mortality.

# 2.5 What Could Be the Approach to Improve Fertility in Dairy Animals?

Having understood the negative impact of high milk production on several physiological pathways, thereby reducing the likelihood of establishment of pregnancy in high yielding cows, the question in front of us is what interventions are needed to improve the fertility in high yielding dairy animals. The interventions may possibly be classified into short- and long-term interventions.

• Long-term intervention: Since selection for milk production with little consideration for reproduction traits in the modern dairy cattle has resulted in an antagonistic relationship between milk yield and reproductive performance, due consideration should be given to traits associated with reproduction while selecting the animals for high milk production.

• *Short-term intervention:* Fertility in dairy animals can be improved using potential reproductive technologies like semen from high fertility sires, controlled breeding, postinsemination fertility enhancement treatments, and assisted reproductive technologies.

#### 2.6 Reproductive Biotechnologies: Development and Applications

Generally, the reproductive biotechnologies are classified into different generations based on the time of development and application (Fig. 2.3). The *first-generation* reproduction biotechnology that has played an unequivocal role in genetic improvement and production enhancement, at least in large animals, is *artificial insemination* (AI) with cryopreserved semen. This technology was born out of the research carried out before 1960s and the progress of this technology has been greatest in several continents. This technology played an important role in animal breeding, especially in the bovines, and brought a visible impact in several countries. The *second-generation* reproduction biotechnology is limited, restricted to few farms and organizations that use this technology for production of elite males and females. The cost of the initial investment in establishing facilities and recurring costs for this technology is higher than that of AI, which explains, at least in part, for its less frequent use. In other parts of the world, this technology is included in genetic



improvement projects and the majority of AI bulls in the world are born out of this technology. The *third generation* reproduction biotechnology, that is, *in vitro fertilization*, is more recent and in use from late 1980s. This technology involves collecting the oocytes very easily at the slaughterhouse as well as collecting the oocytes from live animals through "ovum pick-up." High investments and problems associated with embryo freezing have been the reason for limited use of this technology. However, in considerable number of countries, this technique has really expanded; for instance, Japan and Brazil alone account for about one-half of all the in vitro fertilized and transferred embryos in the world. The *fourth generation* reproduction biotechnology, *nuclear transfer and transgenesis*, is the latest development and mostly restricted to laboratories. The first animal produced by somatic cell cloning was a sheep—"Dolly" and since then – cloning has been carried out in various animals such as cattle, pig, goat, horse, buffalo, and camel.

Other techniques associated with these technologies have immense role in success of the purpose of using these technologies, which include sperm sexing, biomarkers for fertility prediction, gamete cryopreservation, quality improvement of frozen spermatozoa, and fertility improvement protocols. Because the heritability of reproductive parameters is low, application of reproductive biotechniques appears to be an option, which deserves further exploration to inverse the current trend of decreasing fertility in dairy cattle.

### 2.7 Potential Reproductive Technologies Currently Used in Large Scale

*Timed artificial insemination programs:* Maximizing estrus detection accurately and efficiently can improve overall reproductive efficiency in bovines. However, at times, estrus detection by visual observation becomes difficult, because a majority of the animals show the peak estrus activity during night. This leads to difficulties in determination of the actual time of onset of standing estrus, and therefore, the possibilities of performing improperly timed AI are high. Therefore, various estrus induction, estrus synchronization, and timed insemination (TAI) protocols have been developed to improve the conception rate. Also, estrus induction protocols can be applied on postpartum anestrus to bring them into cyclicity so that they can be bred during the breeding period resulting in shorter calving to conception intervals. Generally, prostaglandin F2 $\alpha$  is used to reduce the lifespan of the corpus luteum and to bring the animal into heat. Regularly cycling animals have a functional corpus luteum during the luteal phase of the estrus cycle. Administration of PGF2 $\alpha$  from 5 to 15 days of estrous cycle regresses the active corpus luteum and the animal enters into the follicular phase. PGF2 $\alpha$  is not effective when administered on 0–4 days of the estrous cycle. Generally, the treated animals exhibit estrus within 2–5 days of treatment. The protocols for estrus induction using PGF2 $\alpha$  and timed insemination are given in Table 2.3.

In the recent past, several protocols have been developed and/or modified to allow TAI so as to circumvent the practical difficulties associated with estrus detection.

Day of treatment	Two injection method	One injection method without palpation	One injection method with palpation	
Day 0	$PGF_2\alpha$ injection	$PGF_{2}\alpha$ injection	Rectal examination for presence of CL	
			CL present— PGF <sub>2</sub> $\alpha$ injection	CL absent—No treatment
Day 3 & 4		Breed the animals in heat	Breed the animals in heat	-
Day 11	$PGF_2\alpha$ injection	$PGF_2\alpha$ injection in animals not inseminated	-	CL present— PGF <sub>2</sub> $\alpha$ injection
Day 14 and 15	Insemination at	14th day and /or 15th day (7	72 and 96 h after PG	$F_2\alpha$ injection)

Table 2.3 Estrus induction and timed AI using prostaglandin  $F_{2}\alpha$ 

Some of the protocols have advantage of synchronizing ovulation as well and they can be applied on large scale to improve the fertility in dairy animals. Different protocols are given in Fig. 2.4. Ovsynch protocol is one such, in which gonadotropin-releasing hormone (GnRH) is administered on day 0 of start of treatment (irrespective of the stage of estrous cycle), prostaglandin F2 $\alpha$  is administered on day 7, and again GnRH is administered on day 9. The animal may be inseminated at 16 to 22 h after the administration of second GnRH. The first GnRH injection induces ovulation of the ovarian follicle and subsequent development of the corpus luteum. Depending upon the state of maturation of follicles at the time of GnRH administration, the rate of ovulation induced by the first GnRH injection varies between 65% and 85% (Pursley et al. 1997). The success rate of this protocol is high and being practiced regularly at several farms. A modification of Ovsynch protocol, called as Doublesynch protocol, has been developed by incorporating an additional  $PGF_{2\alpha}$  administration at 48 hour before the start of Ovsynch protocol and claimed to yield superior results. The Controlled Internal Drug Release (CIDR) - GnRH protocol - involves CIDR insertion on day 0 and removal on day 7. GnRH is administered on day of CIDR insertion. On the day of CIDR withdrawal, PGF2 $\alpha$  is administered and after 2 days of PGF2 $\alpha$  administration, the second dose of GnRH is administered. The advantage of inclusion of CIDR in GnRH-based programs is that the animal is under the influence of progesterone during the period between day 0 and day 7, which will prevent early onset of estrus and ovulation. Although all these protocols have been shown to improve fertility, it is important to remember that nutritional status of the animal, sanitary management, and skill of the operator can influence the success rates significantly. Further, it should be remembered that if these protocols are used for all cows without gynecological examinations, some animals may not respond due to undetected reproductive disorders such as true anestrus, ovarian cysts, and subclinical endometritis (Nowicki et al. 2017).

*Multiple ovulation and Embryo Transfer*: In several countries, embryo transfer (ET) technology has played a vital role in genetic improvement of dairy cattle over



Fig. 2.4 Different protocols used for timed AI and synchronization of ovulation with insemination

the past several decades. The advantage of this technology in terms of genetic improvement is not only through the production of cows, but also through the production of bulls for utilization in the artificial breeding program. In USA, 99% and 95% of currently available Holstein and Jersey AI sires, respectively, were produced using ET (Sommer and Youngs 2016). Developments in superovulation protocols and nonsurgical embryo transfer have made MOET technology viable for commercial application. Across the globe, as per the data of International Embryo Transfer Society, a total of 1.41 million cattle embryos (both in vivo derived and in vitro produced) were produced in the year 2019. North America alone accounted for around 52% of the total embryos produced. In recent years, the total number of in vitro produced embryos surpassed the total number of in vivo derived embryos. The increase in the proportion of in vitro produced embryos actually transferred suggests progress in in vitro embryo production systems adopted worldwide. Although such data are not available in several developing countries, existing sporadic reports indicate limited use of MOET in these countries. For instance, in



**Fig. 2.5** Higher number of transferable embryos obtained from a 14-year-old dual purpose cow (Deoni breed). Upper—the donor cow; Bottom—embryos

India, National Dairy Development Board established ET facility in 1987 and ET technology has been used in bull production programs. Similarly, several research and development institutes also started MOET, although not in large scale, and have shown considerable progress. However, the technology needs to be standardized to specific breeds and then implemented at large scale. For instance, it is believed those older cows (>10 years) are generally not to be used for superovulation as the expected embryo recovery is low. However, a recent report from the Southern Regional Station of ICAR-NDRI, Bengaluru, India, shows that MOET was carried out in Deoni breed (a dual-purpose breed of cattle) and obtained 14 transferable embryos (Fig. 2.5) from an old Deoni cow (13.6 years age), indicating that indigenous breeds might have higher oocyte reserves.

In vitro *embryo production and transfer:* An alternate method to superovulation and embryo collection from donor cows is in vitro embryo production. This method involves the steps like collection of the oocytes, in vitro maturation, in vitro fertilization, and in vitro culture of embryos till they reach the stage that they can be transferred to the recipient animals. Oocytes can be collected either from the ovaries obtained from slaughtered animals or from live animals. Ovum Pick-up (OPU) from live animals and in vitro embryo production gained momentum during recent years. OPU is a nonsurgical technique in which oocytes are aspirated from the ovaries of live animals using ultrasound and a guided needle. Combined application of OPU and in vitro embryo production is one of the recent breakthroughs in animal reproduction for improving reproductive efficiency and for quick propagation of elite germplasm. The cow's ovary contains several thousands of oocytes, out of which she may ovulate only about 200 oocytes in her lifetime. Normally, after every estrus, a cow ovulates only one oocyte, but at any given point of time, during estrous cycle, up to 50 antral follicles exist on the ovary. On a conservative estimate, using OPU, 15–20 oocytes can be collected per week from a valuable donor cow, which translates into potential collection of about 700-1000 oocytes/year/cow. Out of these oocytes, 200–300 blastocysts or 80–120 pregnancies can be obtained in a year with the assumption that the blastocyst rate is 30% and a pregnancy rate of 40%. However, to exploit its full potential, a reliable in vitro fertilization system and a dedicated OPU team are needed. As per the data of International Embryo Transfer Society, a total of more than one million embryos were produced using Ovum Pickup and in vitro embryo production in the year 2019.

Use of sexed semen: Normally ejaculates from a bull contains both X chromosome bearing (when take part in fertilization, results in birth of female offspring) and Y chromosome bearing (when take part in fertilization, results in birth of female offspring) spermatozoa. Thus, insemination of a cow with traditional frozen semen straws may result in either female or male calf. However, owing to the developments in techniques and technologies, now, it is possible to separate (sort) X chromosome bearing spermatozoa from Y chromosome bearing spermatozoa. Thus, insemination of a cow with X chromosome bearing spermatozoa-enriched semen is expected to result in birth of female calf. As such, no technology is now available to sort X chromosome bearing spermatozoa from Y chromosome bearing spermatozoa with 100 percent efficiency. Fluorescence-activated cell sorting, a specialized type of flow cytometry, is the only commercial method of sperm sex sorting available as on date and the success rate (in producing desired sex of calf) with this technology is around 90-95 percent. However, several reports indicate that the conception rates with sexed semen are lower than the conception rates with unsexed semen. Efforts are ongoing to minimize the sperm damages associated with the sorting procedure and to enhance the conception rates with sexed semen. By using the sexed semen enriched with X chromosome bearing spermatozoa for artificial insemination (AI), it is possible to substantially increase the number of elite females in short time period. Therefore, artificial insemination using sexed semen is a pragmatic and easy way for preselection of the sex of the offspring. Selective use of sexed semen will increase the genetic progress from the daughterdam path and help in producing superior males from elite bulls for future breeding. Combining MOET/IVEP with sexed semen is further advantageous to multiply superior germ plasm in a shorter time.

#### 2.8 Perspective and Prospective

The ultimate success of the dairy industry depends upon how economically the dairy animals are produced and managed, which in turn is directly influenced by the herd reproductive efficiency. Poor dairy bovine fertility continues to remain as one of the most significant challenges faced by the majority of dairy farmers across the globe. Over the past few decades, significant changes have occurred in dairy bovine production in terms of greater herd size per farm and intense selection of animals for milk production that resulted in increased milk production. To manage high producing bovines, the nutrition and management have changed considerably over the last few decades, but due consideration was not given to the long-term consequences of these nutritional and managemental changes on the basic reproductive physiology of the animal. As a consequence, several studies across the globe have reported a decrease in the fertility of dairy bovine. However, on the other hand, some of the highest yielding and most productive herds in some countries are able to maintain optimum fertility. This clearly indicates that poor fertility is not an inevitable consequence of high milk production in dairy cattle.

Although reproductive parameters have low heritability, by applying careful selection strategy, it is possible to maintain high production as well as reproduction efficiency in dairy animals. Genomic selection combined with advanced reproductive technologies has accelerated the rate of genetic progress in dairy cows. However, the inherent difficulties in selecting animals for high fertility include (i) routinely measured reproductive traits are not very accurate (ii) reproductive traits have low heritability, (ii) animals genetically superior for production tend to have inferior reproduction, and (iv) reproduction traits are polygenic and effect of one gene may depend on others. Mostly days open, cow and heifer conception rate are the three female fertility traits used in genetic evaluations of dairy cattle. Recently, SNPs associated with daughter pregnancy rate, first service pregnancy rate, services per conception, and days open have been identified. However, further research is required to estimate specific parameters for fertility traits and determine whether selection for individual components can affect more rapid genetic change in fertility than direct selection for days open. Recent research identified potential markers for ovarian reserve, cyclicity, conception, fetal growth, calving ease, and postpartum reproductive efficiency. Application of such markers could be valuable in selection of dairy animals for high fertility. On the other hand, the application of reproductive biotechniques within the framework of an adapted reproductive health program could be a viable option to reverse the trend of decreasing fertility in dairy bovines.

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