Review Article

Assisted reproductive technologies and recent developments in *in vitro* embryo production technology in dairy cattle

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Abstract

Scientific and technological researches in livestock reproduction have culminated in the development of various reproductive techniques. Assisted reproductive technologies that include artificial insemination, superovulation, estrus synchronization, *in vitro* fertilization and semen sexing as well as gametes and embryo cryopreservation have a major effect on dairy cattle genetic improvement programs worldwide. The major efforts of assisted reproductive technologies are production of large number of embryos from genetically superior dams and sirs and disseminate genetic material all over the world. To this end, scientific advances in *in vitro* fertilization and embryo production immensely contributed to the genetic improvement in cattle production systems. *In vitro* fertilization with sexed semen has been successfully used to enhance genetic improvement in wider part of the world. Recent developments in reproductive technologies which include ultrasound guided oocytes aspiration, semen and embryo sexing and cryopreservation of embryos immensely contributed for the advancement and success of reproduction and production in dairy industries worldwide.

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Keywords: Assisted reproduction, gametes sexing, *in vitro* fertilization, ovum pickup

Introduction

The uses of assisted reproductive technologies (ART) in animal breeding have become very important tool to the genetic improvement of dairy cattle worldwide. Various reproductive biotechnologies have been invented and refined to speed up genetic progress as well as to obtain a large number of progenies from female donors with superior genetic merit. The ART includes artificial insemination (AI), estrus synchronization, multiple ovulation and embryo transfer (MOET), *in vitro* embryo production (IVEP) (Rodriguez *et al*., 2012) and gamete and embryo cryo-preservation. The introduction and advancement of reproductive biotechnology tools have opened research and experimentation methodologies to manipulate the reproductive phenomenon both *in vitro* and *in vivo* to improve reproductive performance in various domestic species of livestock. Reproductive technologies that are currently in use focus on selective breeding and crossbreeding programs of dairy and beef cattle breeds, that intend to hasten genetic gain from superior dams and sires through accelerating genetic material dissemination and shortening of generation interval.

Differences related to the reproductive physiology among various breed of cattle have been studied for the appropriate use of different reproductive biotechnologies and hence for the establishment of sound reproductive management procedures (Viana *et al.,* 2000). For instance, zebu breeds differ in their reproductive performances in a number of aspects from European breeds of cattle. To this end, it is reported that there are greater number of growing follicles throughout the estrous cycle in zebu cattle. Consequently, more cumulus oocyte complexes (COCs) are recovered using ultrasound guided ovum pic-up (OPU) per oocytes collection session compared to European breeds that accounts to a considerable deference between the breeds (Figueiredo *et al*., 1997; Jemal *et al*., 2020).

Artificial insemination (AI) in animals

Artificial insemination technology has been used for genetic improvement of livestock population by selecting genetically superior bulls that could sire thousands of offspring with rapid genetic progress. Thus, AI technique has immensely hastened the rate of genetic improvement. Preventing the transmission of venereal diseases (Vishwanath, 2003) is also

among the advantages of artificial insemination when compared to natural service (Rodgers *et al*., 2015; Baruselli *et al*., 2017a).

Semen cryopreservation and storage revolutionized AI program through worldwide dissemination and use of semen. The reproductive potential of valuable males has been exploited by combining AI with semen cryopreservation and storage without limitations by time or distance; where a single bull can produce some 50,000 offspring in a year. To this end, AI maximizes the use of outstanding males, dissemination of superior genetic material; improve the rate and efficiency of genetic selection on the male side.

AI also plays an important role in MOET programs. Moreover, AI has contributed immensely in reducing the risk of spreading sexually transmitted diseases (Foote, 2002; ~~Bhoopendra~~ Singh, 2022).

Among the recent advances in assisted reproductive technology is sexing of semen which increases the efficiency of breeding programs in dairy herds. Sex pre-selection is more advantageous for productive purposes than embryo sexing. Sex-sorted bovine sperm can also be used for *in vitro* fertilization (IVF) to generate embryos from in vitro matured oocytes (Cran *et al*., 1993).

The introduction and advancement of sexed semen technology boost the economic viability of IVEP, since the greater proportion of males born after IVF with normal unsorted semen (Camargo *et al*., 2010) was one of the main reasons impairing the economic viability of IVEP in dairy breeds. Though AI has an ample role for the genetic improvement programs; variability in fertility among bulls is still one of the problems influencing the efficiency of this reproductive biotechnology (Seyoum and Lemma, 2022). In spite of the fact that AI has longstanding history and routinely used worldwide, the conception rate from AI programs in developing countries is relatively low and therefore satisfactory results has not been yet achieved. This is mainly attributed to the lack of proper management, particularly inaccurate estrus detection and poor technical skill of insemination (Desalegn *et al*., 2009; Destalem, 2015). According to Debir (2015) AI will become more effective and economically sound when farmers have access to better technical and organizational facilities.

Estrus synchronization and its variants in dairy cattle

Accurate detection of estrus is crucial for good husbandry practice of female cattle. According to Santos *et al*. (2004), reproductive failure in cattle results from poor estrus detection and inappropriate time of insemination. Thus, estrus synchronization has been developed that allow artificial insemination without the need for estrus detection ~~Paul~~ (~~Ashit~~ Kumar *et al*., 2015; Demissie *et al*., 2021). Synchronization of estrus involves manipulation of the estrous cycle or induction of estrus to bring a large proportion of a group of females into estrus at a predetermined time through the use of different hormones including GnRH, $PGF_{2\alpha}$ and progesterone preparations (Santos *et al*., 2004; Demissie *et al*., 2021).

Synchronization protocols allow for the elimination of estrus detection and for the development of fixed time artificial insemination programs. In dairy and beef embryo transfer programs, the said method offer more flexibility in the usage of recipient cows and donors with superior genetic merit (Vasconcelos *et al*., 1999). In line with this, it is proposed that synchronization of follicular wave emergence and of ovulation could increase the success of AI through the use of different synchronization protocols (Vasconcelos *et al*., 1999; Demissie *et al*., 2021). Consequently, various synchronizations protocols have been developed and implemented in dairy cattle industry.

Prostaglandin based estrus synchronization in dairy cattle

Prostaglandin based estrus synchronization protocol uses prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) and its analogues that are luteolytic in female cattle and usually induce estrus when given during the luteal phase of the estrous cycle. Estrus usually occurs within 2 to 6 days of prostaglandin administration in large percentage of a group of treated females (Colazo *et al*., 2002). Indeed, the interval between administration of the hormone and onset of estrus can be affected by the dose and the status of follicular development at the time of prostaglandin administration (Répási *et al*., 2005). Corpus luteum (CL) is not responsive to $PGF_{2\alpha}$ or "refractory "during the first 5 days of luteal development and during the natural regression of corpus luteum in cattle (Levy *et al*., 2006). There is certainly great variability in the interval from treatment to behavioral estrus and ovulation among treated animals when using PGF. In the presence of a responsive CL, estrus can be induced by a single administration of PGF; however, the interval to the resulting estrus

and ovulation is dependent on the stage of development of the dominant follicle at the time of treatment (Kastelic *et al*., 1990a).

A double-injection system of two doses of PGF given at eleven to fourteen days interval from the first administration has been implemented to overcome the problem unresponsiveness of the early and late CL. This method is usually used with an intention that a high proportion of group of animals could have a responsive CL at the time of the second PGF treatment and thus ensures the onset of estrus within few day of injection (Stevenson *et al*., 2007).

GnRH and prostaglandin based estrus synchronization and factors influencing the efficacy

Synchronization of estrus in dairy industry should increase submission rates, improve conception rates, and thus increase the overall pregnancy rate as reviewed in (Lane *et al*. (, 2008). In line with this, the stage of the estrous cycle (Moreira *et al*., 2000) and cyclic status (Bisinotto *et al*., 2010) at the time that gonadotropin releasing hormone (GnRH) is administered has been shown to affect results. Research conducted by Ryan *et al*. (1998) showed that 250g GnRH resulted in ovulation, in 20 of 20 cows, when given at dominance of a follicular wave, this was followed by emergence of a new wave of ovarian follicular growth 1.6±0.3 days later and dominance of the subsequent wave was attained in 5±0.3 days. However, the authors reported that there has been no effect of GnRH on follicular dynamics when given at emergence of a follicular wave. Other works (Martínez *et al*., 2005) indicated that growing follicles greater than 10 mm in diameter ovulate after GnRH injection as administration of GnRH induce massive release of LH and FSH. A new follicular wave is then initiated about two days after the GnRH-induced ovulation. When GnRH treatment is applied before the selection of the dominant follicle, follicular growth is not affected (Ryan *et al*., 1998).

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The low ovulation rate to GnRH given at the early stage of estrous cycle have been related to the fact that protein for LH receptor are not expressed in the granulosa cells of growing follicles during the first two days of the follicular wave. Conversely, the dominant follicle has been found to express LH receptor (Sarkar *et al*., 2009) after the follicular deviation, where the follicles attained ovulatory capacity leading to higher ovulation rate.

During the mid-cycle, there is loss of the functional dominance in the most of the largest follicles of the first follicular wave, increased serum FSH concentrations, and emergence of a new follicular wave (Ginther *et al*., 1996; Vasconcelos *et al*., 1999). When GnRH injection is given

during the late estrous cycle, the percentage of cows that ovulate depends upon whether a new follicular wave is occurring at that time. Pursley *et al.* (1995), recorded 100 % ovulation rate when GnRH administered in the late estrous cycle in a herd of cows where only 2 follicular waves in over 90 % of estrous cycle monitored.

To improve the estrus synchrony, exogenous GnRH, which controls the developmental stage of the pre-ovulatory follicle, has been included with prostaglandin for synchronization of estrus in dairy cows (Thatcher *et al*., 2006). The combinations of GnRH and $PGF_{2\alpha}$ increase the estrous behavior, increase subsequent conception rate and synchronize ovulations leading to a timed artificial insemination (Demissie *et al*., 2021). Other previous works have described a higher rate of estrus synchronization when GnRH is administered 6 or 7d before $PGF_{2\alpha}$ compared to prostaglandin alone (Stevenson *et al*., 2003). These synchronization programs use GnRH and $PGF_{2\alpha}$ to sequentially control ovarian follicular dynamics, luteolysis, and ~~ovulation~~ ~~In~~ovulation. In cows injected with GnRH and $PGF_{2\alpha}$ 7 days apart, detection of estrus should begin 24-48 hours before the $PGF_{2\alpha}$ injection and continued for the next 5-7 days (Demissie *et al*., 2021). The majority of cows exhibited estrus 36 to 72 hours after $PGF_{2\alpha}$ injection (Stevenson *et al*., 2003; Demissie *et al*., 2021). DeJarnette *et al*. (2004) recommend that animals should be inseminated 8 to 12 hours after being observed in standing estrus following the hormone treatment. Such protocol is known as Select-Synch.

Progesterone based synchronization of estrus in dairy cows

The estrous cycle in cows can be controlled by extending the luteal phase by the administration of exogenous progesterone or synthetic progestogens. High level of serum progesterone impedes the final maturation of follicles, inhibits the release of luteinizing hormone and thus suppresses estrus and ovulation.

In the works of (Gümen and Wiltbank, 2005; Cerri *et al*., 2009), progesterone preparations have been used to treat anovular cows and to reduce amount of female cattle showing premature estruses during the timed AI protocol (Rivera *et al*., 2004). Consequently, there have been ~~achieved~~ increased improvements in reproductive performance both in cycling and non-cycling cows treated with different progesterone devices compared to controls (Chebel *et al*., 2010). Gümen and Wiltbank (2005) have revealed that the hypothalamus of lactating cows need to be

exposed to at least 3–5 days of progesterone in order to be responsive to estradiol followed by normal estradiol-induced LH peak release and ovulation.

Progesterone delivery devices; such as progesterone releasing intra-vaginal device (PRID) and controlled internal drug release (CIDR) (Martinez *et al*., 2003) or norgestomet ear implants have been used extensively for synchronization of estrus in cows. Many studies (Lane *et al*., 2008; Sarkar *et al*., 2009) have investigated the addition of progesterone to the Ovsynch or modified Ovsynch protocols. To this end, a CIDR or PRID device is inserted intra-vaginally together with the first injection of GnRH and remains in place, usually for 7 days, until administration of PGF. The use of intra-vaginal devices (CIDR) for controlled release of progesterone from the GnRH to the $PGF_{2\alpha}$ injections maintains blood progesterone concentrations that prevent premature estrous behavior, LH surge and ovulation. These devices have been used during timed AI protocols to improve fertility of dairy cows (EI-Zarkouny *et al*., 2004). Moreover, pregnancy rates were improved, when a CIDR was included in the Ovsynch protocol (59%) compared with Ovsynch alone (36%), as determined by ultrasound 29 days after insemination (Sarkar *et al*., 2009). Likewise, pregnancy rate in cross breed dairy heifers was significantly higher in the group with CIDR Ovsynch (56.3%) than without CIDR (39.3%) as reported in the work of (Demissie *et al*., 2021), who determined pregnancy by ultrasound 32 days post insemination.

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Multiple ovulation and embryo transfer

Multiple ovulation and embryo transfer (MOET) is a method of producing more offspring from a genetically valuable female than would be possible by natural breeding. The aim of MOET programs in the cattle industry is the production of progenies from female cattle of high genetic merit. *In vivo* production of embryos by superovulation also offers a safe and economic way of trading genetic material through cryopreservation. However, the variability in the embryo production of donors and low pregnancy rates after transfer are limiting factors affecting MOET programs (Degefa *et al*., 2016). Some 20% of donors do not respond to the ~~superovulatory~~super ovulatory treatment and thus do not produce embryos. Expected pregnancy rates after transfer are between 50-60%, with best results from fresh or unfrozen embryos, where heifers are considered the best recipients.

MOET has not yet become a widespread tool in developing countries for genetic improvement for various reasons including; costs, technical demands, and variable and unpredictable

efficiency. The use of MOET procedures remains affected by ~~a~~ high variability in the ovulatory response to hormonal treatment and by ~~a~~ low and variable number of transferable embryos and offspring obtained. The reasons for such low efficiency of the technology may be attributed to both extrinsic source, purity of gonadotrophins and protocol of administration, and intrinsic factors such as breed, age, nutrition and reproductive status of the animals (González-Bulnes *et al*., 2004; Degefa *et al*., 2016).

Embryo transfer technology allows producers to obtain multiple progeny from genetically superior females. Fertilized embryos can be recovered from donor females of superior genetic merit by surgical or nonsurgical techniques. The genetically superior embryos are then transferred to recipient females of lesser genetic merit. In cattle and horses, efficient techniques recover fertilized embryos more commonly using non-surgical method of embryo collection.

MOET techniques contribute towards faster improvement of livestock population, rapid expansion of elite animals, genetic gain, accelerated herd development and conservation of rare genetic stocks. Intrinsic factors related to the donor and the recipient animals need to be taken into account when applying such a technology. In addition, environmental factors also play a pivotal role in the success of the reproductive technology, especially under tropical conditions. Considerable progress has been made in the improvement of the outcome in MOET programs (Bergstein-Galan *et al*., 2019).

The emerging and advancement of reproductive biotechnology techniques have wide opened research and experimentation opportunities to manipulate the reproductive events both *in vivo* and *in vitro* to improve reproductive performance in various domestic species of livestock. Conventional embryo transfer technology with the combination of *in vitro* embryo production has proved its advantage to assist and hasten the advancement of genetic progress from elite dams and balance the female side of the equation for genetic progress (Viana, 2018). According to Viana (2017) more than 1.6 million *in vivo* produced and IVF embryos were transferred worldwide in 2017.

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In vitro embryo production (IVEP)

In vitro embryo production (IVEP) involves collection and *in vitro* maturation of the oocytes (IVM), *in vitro fertilization* (IVF) and in vitro culture (IVC) of embryos up to a stage that is compatible to microenvironment of the recipient uterus at the time of transfer (Freitas and Melo, 2010). The procedures have progressively advanced into successful commercial applications. The advancement of these techniques have been playing a pivotal role in increasing the production through genetics, reduction in generation intervals, extending reproductive lifetime of female animals, controlling of diseases, and the reduction in production costs (Verma *et al.*, 2012).

The development of embryo technologies have been driven by the economic gain offered by the potential increase in the number of offspring from genetically superior animals that provide the possibilities for broader use of superior genetic material.

In the work of Lonergan and Fair (2016), large-scale production of embryos in vitro has been possible in domestic mammals, particularly cattle, for purpose of generating large numbers of embryos for research, commercial or as a source to other genetic engineering technologies, such as nuclear transfer and trans-genesis. Currently, bovine IVEP is well-established and proven to be an efficient procedure for genetic improvement. Transvaginal Ovum Pick-Up (OPU) in combination with *in vitro fertilization* has shown its worth in increasing the yield of embryos from female donors of superior genetic merit. Moreover, IVEP can also be used to salvage irreplaceable genetic material following slaughter for controlling of infectious disease and culling for other reasons (Hasler, 2003).

Oocytes can be harvested from ovaries of both slaughtered and live female donors for the *in vitro* production of bovine embryos. Recovery of oocytes from live animals is commonly conducted using transvaginal ultrasound-guided oocyte collection method (Pieterse *et al.*, 1988). Ultrasound guided transvaginal ovum pick up (OPU), in conjunction with *in vitro* embryo production (IVEP), provides an alternative means to increase the number of offspring from genetically valuable cows. The technique overcomes some shortcomings of superovulation and the use of ovaries obtained from slaughterhouse for *in vitro* embryo production (Xiao-Yu Yang *et al.*, 2007). OPU is the most flexible and repeatable technique to produce embryos from live donor females in their different stage of reproductive life. It can also be applied in female cattle

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with genital tract illness that do not respond or with low sensitivity to super ovulatory treatments. The application of this technique to female calves (Yang *et al*., 2008) has also proved its advantage in accelerating genetic gain by considerably decreasing the generation interval (Donnay, 2004).

The frequency of ultrasound guided oocyte retrieval from ovaries of female cattle has effect on the number and quality of the cumulus oocyte complexes (COCs) and subsequent culture procedures (Imai *et al*., 2006).Various oocyte collection patterns have been adopted for OPU where once and twice a week follicular aspirations are the major schemes based on the interval between follicular punctures (Jemal *et al*., 2020; Demissie *et al*., 2021b).

The greater proportion of males calves born after *in vitro fertilization* with conventional unsorted semen has been one of the main reasons impairing the economic feasibility of IVEP in dairy breeds (Camargo *et al*., 2010). However, the introduction and advancement of sexed semen technology boost the economic viability of IVEP.

Sex-sorted semen is used in cattle breeding that in turn enabled to effectively control the sex ratio of the offspring of dairy cows (Seidel and Schenk, 2008; Seidel, 2012). Using sexed semen for *in vitro* fertilization requires less sperm cell number than normal AI, but sexed semen typically results in low fertilization, cleavage, blastocyst and pregnancy rates (Lonergan, 2007; Lonergan and Fair, 2008).

Sources of oocyte for in vitro embryo production

Bovine oocytes could be harvested from slaughterhouse ovaries of any age females and using the method of ultrasound guided ovum pick-up (OPU) (Machaty *et al*., 2012). The oocytes obtained from slaughterhouse ovaries of cattle have potential of high developmental competence to produce large numbers of embryos which can result in live calves through IVF procedures (Natumanya *et al*., 2008). Slaughterhouse derived ovaries contain heterogeneous population of oocytes that are recovered regardless of follicular dynamics and are variable in their developmental competence in *in vitro* maturation medium (Bilodeau and Panich, 2002).

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Oocytes can be recovered from ovaries of slaughtered cows by using aspiration, slicing or puncturing (Abid *et al*., 2011). The interval between animal slaughter to the oocyte recovery from the ovaries and the temperature at which the ovaries should be stored are important factors that need consideration. The technique is cheap and allows harvesting of all follicles visible on

the surface of ovaries. To this effect, *in vitro* production of embryo in animals is mostly dependent on a supply of slaughterhouse ovaries; it is a source of oocytes of large-scale production of average genetic merit embryos (Dadashpour *et al*., 2014).

Ultrasound guided transvaginal oocyte recovery in IVEP has proven to be a valuable development in the cattle breeding and embryo transfer industry (Duszewska and Reklewski, 2000; Saini *et al*., 2015). The technique is a non-invasive and flexible procedure for collecting oocytes ranging from small to large follicles in live animals (Demissie *et al*., 2021b). Valuable donors at different stages of reproduction and donors that are sub-fertile can be included in the follicular aspiration with a frequency of once or twice per week and with or without hormonal stimulation (Chaubal *et al*., 2006). Ultrasound guided ovum pick-up also help recovering oocytes from young heifers, pregnant cows in their first trimester and from cows during the early post-partum period (Perez *et al*., 2000). Such collection schemes can be carried out for longer periods, where recovery of about four to five quality oocytes per each collection session can be achieved (Galli *et al*., 2001). According to ~~Mufeed A.~~ Alnimer (2005), a number of factors such as breed of cattle, age of ~~the~~ donor, number of follicles present, ~~the~~ frequency of collection, and ~~the~~ vacuum pressure used to retrieve the follicular fluid, ~~the~~ size and type of ~~the~~ aspirating needle affect the number of intact COCs collected (Hasler *et al*., 2015). Manipulation of follicular dynamics (Ferré *et al*., 2020) and function through hormonal treatment and dietary supplementation with energy concentrates that are rich in fatty acids (Dunning *et al*., 2014) would help to improve oocyte quality within the follicle in OPU-IVF programs. They have several advantages over conventional MOET; up to 80 to 120 live calves could be born per donor per year using these techniques (Reviewed by Van Wagtendonk de Leeuw, 2006).

In vitro oocyte maturation

In vitro oocyte maturation is a process in which meiotically-arrested oocytes from small to medium antral follicles are cultured in the laboratory to become ready for fertilization. The oocytes become matured in maturation medium which is determinant for the subsequent fertilization and further development (~~Bilal~~ Alfoteisy *et al*., 2020). Bovine oocytes are matured at 39.0°C, 5% CO_2 in air and high humidity for 22-24h (Prentice-Biensch *et al*., 2012). A commonly used media for bovine oocyte maturation is M199 medium (Ferré Luis *et al*., 2016).

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M199 contains antioxidants which are missing from TALP and SOF media formulations. Antioxidants are important in mitigating the accumulation of reactive oxygen species (ROS) that can generate oxygen stress and damage normal physiological sperm function (Bansal and Bilaspuri, 2010; Aitken *et al*., 2012), impair sperm motility, affect membrane integrity and decrease oocyte penetration capacity (Bansal and Bilaspuri, 2010; Aitken and Henkel, 2011).

Oocyte maturation coincides with expression of estrus in the cow and is important to prepare the ovum for *in vivo* fertilization. Maturation of oocytes is one of the most determinants of subsequent embryo quality. It is the most important stage, which determines the subsequent successful fertilization, zygote formation, attainment of blastocyst stage and normal embryo growth and development (Ward *et al*., 2002). Thus, the production of competent oocytes during *in vitro* maturation (IVM) is crucial to increase production of valuable, healthy offspring in cattle reproduction (Rizos *et al*., 2002). During IVM, the oocyte undergoes processes that are crucial not only for the activation of the embryonic genome, but also throughout development to the blastocyst stage. Changes occurring in the nucleus and cytoplasm are referred to as nuclear and cytoplasmic maturation, respectively.

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Nuclear maturation

Nuclear maturation refers to the progression of ~~the~~ oocyte nucleus from ~~the~~ germinal vesicle to ~~the~~ Metaphase-II (MII) stage. The maturation process is related to RNA synthesis activity, characterized by nucleus changes from ~~the~~ diplotene phase to metaphase II (~~Herry~~ Sonjaya and ~~Hasbi~~ Hasbi, 2019). Nuclear maturation mainly involves germinal vesicle breakdown, condensation of chromosomes, metaphase-I spindle formation, separation of the homologous chromosomes with extrusion of the first polar body and arrest at MII stage. It initiates germinal vesicle break down, ~~the~~ separation of homologue chromosomes and ~~the~~ extrusion of the first polar body (Grondahl, 2008; ~~Herry~~ Sonjaya and ~~Hasbi~~ Hasbi, 2019).

Cytoplasmic maturation

Cytoplasmic maturation involves the ultra-structural changes that take place in the oocyte from the GV to the MII stage. Protein synthesis and transcription of cytoplasmic RNA occur in the oocyte cytoplasm from the germinal vesicle (GV) stage to the end of MII in which the

developmental competence of the oocyte is attained (Hyttel *et al*., 1997; Duranthon and Renard, 2001). The ultra-structural changes in the cytoplasm include migration of a number of the organelles. Cytoplasmic maturation is indirectly assessed as the ability of the mature oocyte to undergo normal fertilization, cleavage and blastocyst development. Cumulus cell expansion, extrusion of the first polar body and an increased perivitelline space are other indirect morphological parameters that can be taken into account to evaluate cytoplasmic maturation (Hendriksen *et al*., 2000).

Maturation of follicular oocytes is normally arrested at the prophase-I of the first meiotic disappears and germinal vesicle breaks down followed by chromosome condensation division and the oocyte remain in the dormant stage. At this stage, nuclear material is enveloped and the immature oocyte is referred as germinal vesicle. Upon extrusion of the first polar body, the oocytes reach the Metaphase-II stage and remain at this stage until penetration by the spermatozoa (Kharche *et al*., 2006). In addition, the cumulus cells expand in response to a changing environment of gonadotropins, growth factors, steroid hormones, and other factors that become secreted by the oocyte during maturation (Buccione *et al*., 1990), the oocytes must reach its nuclear and cytoplasmic maturation before fertilization occurs.

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The presence of cumulus cells is required for oocyte maturation (Shirazia *et al*., 2007), as interce llular communication between oocyte and cumulus cells is important for the acquisition of developmental competence (Boni *et al*., 2002; Kidder and Vanderhyden, 2010). For this reason, oocytes surrounded by several layers of compact cumulus cells are selected for IVM (Massip, 1995; Boni *et al*., 2002). During culture, expansion of the cumulus cells is a visible sign of oocyte maturation and is used as a predictor of the developmental competence of oocytes matured *in vitro* (Miriam *et al*., 2016).

The cumulus cells expand in response to a changing environment of gonadotropins, growth factors, steroids, and certain other factors that become secreted by the oocyte during maturation (Buccione *et al*., 1990). To this end, it noted that optimal environment is essential to maximize the number of mature oocytes after a period of 22 hours, which requires 39.5°C, 5% CO_2, highest possible humidity, and a suitable culture media that meets the optimal nutritional and hormonal requirements. After maturation, the oocytes are ready for fertilization.

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Semen preparation for in vitro fertilization

Sperm preparation for *in vitro fertilization* involves procedures that enable to separate spermatozoa from seminal plasma, extender and cryo-protectant (CPA). Sperm selection is essential to obtain spermatozoa of good quality and high density from frozen-thawed semen for *in-vitro fertilization* (IVF). Most spermatozoa are damaged during semen freezing and thawing processes, leading to a low motility percentage and damaging of membrane structures (Hae-Lee Lee *et al*., 2009; Samardzija *et al*., 2015). The number of sperm added to oocytes during IVF impacts the percentage of oocytes penetrated by sperm (Parrish, 2014). In fresh semen, most sperm cells are motile, determining the concentration and adding a certain amount of sperm to *in vitro* fertilization plate may be sufficient. Conversely, in frozen-thawed semen, many sperm die in the cryopreservation process resulting in post-thaw motilities of 30-70% (Parrish *et al*., 1995). There are various methods that are used in the preparation of sperm for *in vitro* fertilization procedure (Oliveira *et al*., 2011), where Percoll gradient centrifugation and swim-up are the most used methods to obtain high-quality sperm with good progressive motility and intact structures (Parrish *et al*., 1994; Samardzija *et al*., 2015).

Swim-up method

Swim-up is a method that routinely used to separate motile from non-motile spermatozoa in vitro whereby sperm cells are allowed to swim upwards through an overlaid medium. In swim-up, sperm are layered at the bottom of a column of medium, the dense nature of semen in extender and cryo-protectant (CPA) initially keeps the sperm cells at the bottom. Over time motile sperms with normal morphology begin to swim-up out of the extender and CPA into the covering or upper medium. Sperms that swim up into the upper medium display increased motility, higher average velocity, higher percentage of normal morphology and generate improved fertilization rates *in vitro* in mammals (Yavetz *et al*., 2018). Non motile, dead and abnormal sperms remain at bottom. The swim-up isolated population of sperm can be counted in order to add a specific number of sperm to the IVF system.

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Percoll density gradient method

Percoll density gradient is one of the commonly used methods for separating spermatozoa and the most widely used in bovine IVF laboratories (Cesari *et al*., 2006). Sperm preparation by density gradient separates sperm cells based on their density. A mature morphologically normal spermatozoon has a slightly higher density whereas an immature and morphologically abnormal spermatozoon has a lower density (Allamaneni *et al*., 2005). Percoll comprises colloidal silica particles covered with polyvinylpyrrolidone; is prepared in different concentrations to form a discontinuous gradient of two or three phases (90, 45 and 30%) for separating sperm (Gonsalves *et al*., 2002). Frozen-thawed sperm cells are selected by centrifugation on a Percoll discontinuous gradient (45% and 90%). The 45% Percoll solution is prepared with equal amounts of 90% Percoll and sperm washing medium such as tyrode-albumin-lactate-pyruvate (TALP). After centrifugation the resulting interphases between seminal plasma and the 45% upper layer, containing the leukocytes, cell debris and 90% containing morphologically abnormal sperm with poor motility are discarded. Highly motile, morphologically normal, viable spermatozoa form a pellet at the bottom of the tube (Henkel *et al*., 2003; Machado, 2009; Malvezzi *et al*., 2014). Parrish *et al*. (1995) made a comparison of the Percoll approach for sperm isolation with the swim-up method and found a recovery of motile sperm from frozen thawed semen by swim-up approach as 9% and 40% for Percoll approach. Chen *et al*. (1998) reported a recovery rate of motile bovine sperm after Percoll gradient treatment as 58% in comparison with 16% for sperm recovered by swim-up. Percoll procedures were adopted in IVF laboratories due to higher viable sperm recovery.

In vitro fertilization

Fertilization is not a simple process rather it is a multifactorial and complex process that includes sperm-ovum interaction, fusion and initiation of embryo development. The process of *in vitro* fertilization includes sperm capacitation, and sperm binding to oocytes (Lessard *et al*., 2011), followed by acrosome reaction, penetration of zona pellucid, binding and fusion of sperm with oocyte plasma membrane, introduction of paternal hereditary material into the oocyte and ultimately leads to the fusion of male and female chromosomes (Lessard *et al*., 2011).

In vitro fertilization, fusion of oocyte and sperm cell is very complex process that requires competent oocytes and sperm cells as well as optimal culture conditions. The success of *in vitro* fertilization can be influenced by appropriate oocyte maturation, sperm selection, sperm capacitation and IVF media (Garcia *et al*., 2016). Different fertilization media have been used with Tyrode's Albumin-Lactate-Pyruvate (TALP) (Hammami, 2014) being the most commonly used. TALP is designed with a specific ionic balance for oocyte and sperm requirements. To induce sperm capacitation TALP was supplemented with heparin (Parrish, 2014).

Sperm-oocytes co- incubation time of 10hr is needed to ensure maximal blastocyst yields, whereby spermatozoa cross all physiological barriers, fuse with the ooplasma, the oocyte is activated and the pronuclear formation begins (Galli *et al*., 2003). Ward *et al*. (2002) demonstrated that blastocyst development was maximized when IVF was performed at 24 IVM and performing IVF earlier (at 16 to 20h IVM) or later (at 28 to 32h IVM) reduce blastocyst development. The actual fertilization takes between 6 to 20 hours and requires a constant temperature of 39°C and an atmosphere that is enriched with 5% CO_2. The success of *in vitro* fertilization of bovine oocytes is estimated to be 48 hours following fertilization by a number of cleaved embryos and by identification of male and female pronuclei which may be visible after 18-22 hours of co-incubation (Leibfried-Rutledge *et al*., 1996).

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In vitro embryo culture

After fertilization embryos are incubated for 6 – 7 days *in vitro* until the blastocyst stage is attained, when embryos are intended to be transferred into a recipient or frozen and stored in liquid nitrogen (Samardzija *et al*., 2015). Zygotes are cultured in a defined culture medium where embryos are passing through different developmental stages during the *in vitro* culture period. Embryos are cultured at 39^0C, 5% CO_2 under atmospheric air in desirable defined sequential media to satisfy energyetic needs of the pre-implantedation embryo (Samardžija *et al*., 2005). In *in vitro*, the pre-implantation development of the embryo occurs in close relation with the culture medium that demands specific balanced conditions in the medium to allow embryonic metabolism (Boni *et al*., 2002; Samardzija *et al*., 2015). Synthetic oviduct fluid supplemented with bovine serum albumin and essential and non-essential amino acids yielded relatively constant developmental rates of embryos (Gardner, 1994). During in vitro culture (IVC), embryos are passing through significant developmental stages including mitosis cell division,

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activation of embryonic genome, compaction in morula stage, and ~~the~~ formation of the blastocyst with further differentiation of the cells (Samardzija *et al*., 2015). The quality of the embryo in turn influences the success of freezing as well as conception rate after transferring to the recipient animal.

Following *in vitro* maturation, approximately 90% of immature bovine oocytes will reach metaphase II and extrude the first polar body; approximately 80% will undergo fertilization and cleave, at least once, to the two-cell stage. However, only about 30–40% will ever reach the blastocyst stage. This would suggest that the post-fertilization part of the process of *in vitro* embryo production, the longest part, is the main period determining blastocyst yield (Lonergan and Fair, 2016). Rizos *et al*. (2003) indicated that post-fertilization culture environment is critical in determining blastocyst quality, measured in terms of cryotolerance and relative transcript abundance irrespective of the origin of the oocyte. Similarly, Galli *et al*. (2003), Hasler (2003), Lonergan (2003) demonstrated that pregnancy rates after transfer of both frozen–thawed and fresh *in vitro* produced embryos are generally significantly reduced even under commercial conditions which might suggest that post fertilization embryo culture is the most critical period.

Recent developments in in vitro embryo production technology

Scientific advances in *in vitro* embryo production (IVEP) are immensely contributed for genetic improvement in cattle production systems. The combination of *in vitro* embryo production with sexed semen and genomic selection has been successfully used in North America, South America and Europe (Ferré *et al*., 2020). The main advantages offered by these technologies include a higher number of embryos and pregnancies per unit of time, and a wider range of potential female donors from which oocytes are retrieved (Ferré *et al*., 2020).

There are many facts in the process of *in vitro* embryo production and transfer that are subjected to manipulation for improvement of success. To this end, *in vitro* productions (IVP) of embryos and associated technologies in cattle that have shown significant progress in the recent years.

Ultrasound guided transvaginal follicular aspiration

Collecting oocytes from live animals involves puncturing and aspiration of ovaries manipulated per rectum and guided by a vaginally inserted ultrasound probe and needle. Transvaginal

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ultrasound-guided oocyte aspiration techniques allow for non-invasive recovery of oocytes from genetically valuable animals.

Previously conducted repeated collection oocytes via flank incision from living donor cows resulted in high recovery rates but were expensive, inefficient, and risked the formation of adhesions with subsequent loss of fertility (Lambert *et al*., 1986; Hasler *et al*., 2015). In the contrary, repeated transvaginal follicular aspiration sessions can be performed in cattle without significant side effects and with a minimal stress to the animal (Velez *et al*., 2012). The most significant features of the success *in vitro* embryo production systems are quality of the determinant materials, ~~the~~ ovum and sperm. Oocyte quality is a determining factor for the subsequent developmental competence of the embryo (Singh, 2009). The introduction and advancement of ovum pick-up and IVF techniques in cattle, IVEP has been playing a paramount role for genetic improvement and expansion for dairy industry (Galli *et al*., 2014).

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Semen sexing

Numerous successful discoveries in biological sciences, including identification of the sex chromosomes were achieved in the first half of the 20th century in which differential density (Manual on Assisted Reproduction, 2000) of X and Y sperm in Rabbit was identified (Reviewed by Hanuman Prasad Yadav *et al*., 2018). Semen sorting technology separates X and Y chromosome bearing sperm by measuring differences in fluorescence following staining the sperm with a non-toxic, DNA-binding dye (Hoechst 33342) (Reviewed by Hanuman Prasad Yadav *et al*., 2018). Despite reliable separation of X- and Y chromosome-bearing sperm based on X/Y DNA content deference at a purity of 95%, ~~the~~ fertility of the sexed semen product is compromised (Reviewed by Van Wagtendonk de Leeuw, 2006). Following the initial development of ~~the~~ flowcytometric method of sex-sorting semen (Garner *et al*., 1983), numerous technical advances have enhanced the throughput and the sorting efficiency of semen sorting process (Hanuman Prasad Yadav *et al*., 2018). Currently, sexed semen is widely used in dairy industries all over the world and is nearing commercial application in several other species (Seidel, 2012).

Embryo sexing

In vitro ~~M~~manipulation of embryos to determine the sex ratio prior to transfer is an alternative reproductive technology, particularly in the dairy industry. In mammals, sex is determined genetically by the presence of the SRY gene (sex-determining region on the Y chromosome), which encodes the testis-determining factor (TDF) on the Y- chromosome (Koopman *et al*., 1991).

Embryo sexing methods can be grouped in to invasive and non-invasive techniques. The non-invasive method of embryo sexing is generally considered optimal, since embryonic integrity is maintained and the capacity for normal development should be optimized. More rapid, reliable and non-invasive embryo sexing techniques such as PCR (Polymerase Chain Reaction) and fluorescent *in-situ* hybridization (FISH) are being evolved with recent progress in molecular biology (Jafar and Flint, 1996; Sharma *et al*., 2019). A routine method of embryo sexing must include an accurate sexing procedure and survival of embryos. Sexing methods must also be easy to use and inexpensive to operate.

Embryo sexing employs methods ~~such as~~are, immunological assays of male-specific antigens (H-Y antigen), quantification of the X-linked enzymes and analysis of sex chromatin with Y-specific DNA probes and Karyotyping/Cytogenetic analysis. Cytogenetic analysis and the use of Y specific probes are highly accurate, but they are invasive methods that are limited by the biopsy (Jafar and Flint, 1996). The recent development of the polymerase chain reaction allows amplification of Y- chromosome-specific repetitive sequences and thus determination of the sex of the embryo in a relatively short time and with high reliability (Herr Charles *et al*., 1990).

Cryopreservation of embryos

The aim of embryo cryopreservation is preserving of cells in quiescent state, prolong viability, and enabling the use of cryopreserved cells in a timely manner. Cryopreservation of embryos is the process of freezing and storing embryos and an integral part of genetic improvement programmes utilizing embryo transfer and also is vital to germplasm preservation programmes. Pre-implantation embryos are preserved and stored in liquid nitrogen at -196^{o}C. The

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advancement of embryo cryopreservation in domestic mammalian livestock species has been reviewed by Youngs *et al*. (2010). Despite recent advancements in embryo cryopreservation, bovine embryos remain very sensitive to chilling and cryopreservation (Ferré *et al*., 2020; Naitana and Ledda, 2020). The poor cryo-tolerance of *in vitro* produced embryos is a limiting factor to the cryopreservation practice in IVF program (Sudano *et al*., 2011).

There are two methods for bovine IVP embryo cryopreservation; slow programmable freezing and vitrification (Rezazadeh Valojerdi *et al*., 2009). Slow freezing is widespread embryo cryopreservation technique and is based on the use of glycerol or ethylene glycol as a CPA. The main advantage of this technique is the reduced cellular toxicity through the use of low concentrations of CPA; however, slow freezing still allows the formation of ice crystals that could lead to cellular damage. Vitrification method of cryopreservation enables passage from the liquid to the solid state by extreme elevation of viscosity due to high concentration of cryoprotectants and very rapid cooling. Vitrification eradicates the damage caused by the formation of ice crystals during the cooling process. The cryopreservation medium undergoes a direct passage from the liquid state to a vitrified and amorphous state without the crystallization of the medium occurring, which is possible due to the high viscosity of the cryopreservation medium and the high freezing rate by direct immersion in N2, from room temperature (~~Rezazadeh~~ Valojerdi *et al*., 2009; Naitana and Ledda, 2020).

Massip (2001) reported that there are differences not only between species at the same stage of development of embryos but also in the same species all stages of development do not survive equally under the same freezing protocol. Cattle oocytes and early stages of embryo development *in vivo* or *in vitro* do not survive whereas compacted morulae and blastocysts survive very well. In ~~the~~ pig, hatched blastocysts survive better than the other stages. Horse embryos have special characteristics that pose problems for successful freezing.

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Conclusion

The uses of assisted reproductive technologies (ART) in animal breeding have become very important tool to the genetic improvement of dairy cattle worldwide. Production of increased number of genetically improved progenies, a reduced number of sperm required to produce embryos and increased chances of obtaining the desired sex of offspring are among the achievements of the recent advancement in the reproductive technologies.

References

Abid M, Muhammad A, Sayed M, et al., 2011. In vitro maturation and fertilization of buffalo oocytes:the effect of recovery and maturation methods. Turk J Vet Anim Sci. 2011;35(6):381-386.

Aitken R J, Jones KT, Robertson S A, 2012. Reactive oxygen species and sperm function: in sickness and in health. Journal of Andrology, 33:1096 1106. doi:10.2164/JANDROL.112.016535

Aitken RJ, and Henkel RR, 2011. Spermcellbiology:currentperspectives and future prospects. Asian Journal of Andrology, 13: 3–5.doi:10.1038/AJA.2010.155

Allamaneni SS, Agarwal A, Rama S, et al., 2005. Comparative study on density gradients and swim-up preparation techniques utilizing neat and cryopreserved spermatazoa. Asian J Androl, 7:86–92

Bansal A K, and Bilaspuri, G S, 2010. Impacts of oxidative stress and antioxidants on semen functions. Veterinary Medicine International, 10: 1-7.doi:10.4061/2011/686137

Baruselli PS, Ferreira RM, Colli MHA, et al., 2017a. Timed artificial insemination: current challenges and recent advances in reproductive efficiency in beef and dairy herds in Brazil. Animal Reproduction, 14: 558-571.

Bergstein-Galan TG, Weiss RR, Kozicki LE, 2018. Effect of semen and donor factors on multiple ovulation and embryo transfer (MOET) in sheep. Reprod Domest Anim. 2019 Feb;54(2):401-407. doi: 10.1111/rda.13381. Epub 2018 Dec 10. PMID: 30472758.

Bhoopendra Singh, Kaushlendra Pratap Singh, Mozammel Hoque, et al., 2022. "Biotechnology in livestock reproduction", Elsevier BV,

Bilal Alfoteisy, Jaswant Singh, Muhammad Anzar, 2020. "Natural honey acts as a nonpermeatingcryoprotectant for promoting bovine oocyte vitrification", PLOS ONE, 2020

Bilodeau-Goeseels, S, P Panich, 2002. Effects of oocyte quality on development and transcriptional activity in early bovine embryos. Anim. Reprod. Sci. 71, 143 155. 64

Bisinotto RS, Chebel RC, Santos JEP, 2010. Follicular wave of the ovulatory follicle and not cyclic status influences fertility of dairy cows, Journal of Dairy Science, 93: 3578-3587 DOI:https://doi.org/10.3168/jds.2010-3047

Boni R, Cuomo A, Tosti E, 2002 Developmental potential in bovine oocytes is related to cumulus- oocyte complex grade, calcium current activity, and calcium stores. Biol Reprod, 66:836– 42.

Buccione R, Schroeder AC and Eppig JJ, 1990. Interactions between somatic cells and germ cells throughout mammalian oogenesis. Biology Reproduction, 43(4): 543-7.

Camargo LSA, Freitas C, As WF, et al., 2010. Gestation length, birth weight and offspring gender ratio of in vitro-produced Gyr (Bos indicus) cattle embryos. Animal Reproduction Science, 120: 10-5. 11

Cerri RLA, Rutigliano HM, Chebel RC, et al., 2009. Period of dominance of the ovulatory follicle influences embryo quality in lactating dairy cows. Reproduction, 137:813-823.

Cesari A, Kaiser GG, Mucci N, et al., 2006. Integrated morphophysiological assessment of two methods for sperm selection in bovine embryo production in vitro, Heriogenolog\ 66: 1185-1193.

Chaubal SA, Molina JA, Ohrichs CL, et al., 2006. Comparison of different transvaginal ovum pick up protocols to optimize oocyte retrieval and embryo production over a 10 week period

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in cows. Theriogenology, 65: 1631–1648. https://doi.org/10.1016/j.theriogenology.2005.07.020
Chebel RC, Al-Hassan MJ, Fricke PM, et al., 2010. Supplementation of progesterone via controlled internal drug release inserts during ovulation synchronization protocols in lactating dairy cows. Journal of Dairy Science, 93:922–31. DOI: 10.3168/jds.2009-2301
Chen SJ, Liang Guan Sheng, Zhang Jia Bao, et al., 1998. Effect of bovine sperm separation by the Percoll method on the develoipment of embryos fertilized in vitro. Chinese Journal of Veterinary Science, 18:394-397.
Colazo M G, Martínez M F, Kastelic J P et al., 2002. Effects of dose and route of administration of cloprostenol on luteolysis, estrus and ovulation in beef heifers. Animal Reproductio Science, 72: 47–62.
Cran D G, Jhonson LA and Miller N GA, 1993. Production of bovine calves following sepration of X- and Y- bearing sperm and invitro fertilization. Vet. Rec. 132, 40-41.
Dadashpour Davachi, N, Zare Shahneh, A, Kohram, et al., 2014. In vitro ovine embryo production: the study of seasonal and oocyte recovery method effects. Iranian Red Crescent medical journal, 16(9), e20749. https://doi.org/10.5812/ircmj.20749
Debir L, 2015. Assessment of breeding practice and evaluation of estrus synchronization of dairy cattle in Sidama zone, Southern Ethiopia. MSc Thesis, Hawassa University Ethiopia.
Degefa T, Lemma A, Jemal J, et al., 2016. Ovarian follicular dynamics in purebred and crossbred Boran cows in Ethiopia, African Journal of Biotechnology, 15 (33): 1763-1770.
DeJarnette, JM, RB House, WH Ayars, et al., 2004. Synchronization of estrus in postpartum beef cows and virgin heifers using combinations ofmelengestrol acetate, GnRH and PGF2α. J. Anim. Sci. 82:867-877 (5) _GnRH
Demissie T, Yilma T, Degefa T, et al., 2021b. Effect of follicular ablation and gonadotropin priming on the recovery and quality of oocytes in Boran cows. Int J Vet Sci Res 7(2): 138-143. DOI: https://dx.doi.org/10.17352/ijvsr.000093
Demissie Tilaye, Tefera Yilma, Tamrat Degefa, et al., 2021. Pregnancy Rate of Cross Breed Dairy Heifers Subjected to Ovulation Synchronization and Fixed Time Artificial Insemination. Journal of Animal and Veterinary Advances, 20: 86-90. DOI: 10.36478/javaa.2021.86.90
Desalegn G, Merga B, Azage T, et al., 2009. Status of artificial insemination service in Ethiopia. In: The 17th Annual Conference of the Ethiopian Society of Animal Production (ESAP), Sept 24-26, 2009. Addis Ababa, Ethiopia, 87-104.
Destalem G, 2015. Breeding Practice and Estrus Synchronization Evaluation of Dairy Cattle in Central Zone of Tigray, Northern Ethiopia. M.Sc. Thesis Jimma University
Donnay, I, 2004. "Effect of prematuration, meiosis activating sterol and enriched maturation medium on the nuclear maturation and competence to development of calf oocytes", Theriogenology, 20040915
Dunning KR, Russell DL, Robker RL, 2014. Lipids and oocyte developmental competence: the role of fatty acids and β-oxidation. Reproduction. 2014;148:R15–R27. doi: 10.1530/REP-13-0251.
Duranthon V, Renard JP, 2001. The developmental competence of mammalian oocytes: a convenient but biologically fuzzy concept. Theriogenology. 55(6):1277-89. doi: 10.1016/s0093-691x(01)00482-4. PMID: 11327684.
Duszewska AM and Reklewski Z, 2000. Reproduction of cattle using the OPU-IVP-ET. Prace I Materialy Zootechniczne, 57: 111-120.

El-Zarkouny, SZ, JA Cartmill, BA Hensley et al., 2004. Presynchronization of estrous cycles before Ovsynch andprogesterone in dairy cows: Ovulation, pregnancy rates, and em-bryo survival. J. Dairy Sci. 87:1024–103.

Ferré LB, Kjelland ME, Strøbech LB, et al., 2020. Review: Recent advances in bovine in vitro embryo production: reproductive biotechnology history and methods. Animal, Cambridge University Press: 14: 991-1004. DOI: https://doi.org/10.1017/ S1751731119002775

Ferré Luis B, Yanina Bogliotti, James L, et al., 2016. "Comparison of different fertilization media for an in vitro maturation–fertilisation–culture system using flow-cytometrically sorted X chromosome-bearing spermatozoa for bovine embryo production", Reproduction, Fertility and Development, 2016

Figueiredo RA, Barros L M, Pinheiro OL et al., 1997. Ovarian follicular dynamics in Nellore breed (Bos indicus) cattle. Theriogenology, 47: 1489-1505.

Foote RH, 2002. The history of artificial insemination: Selected notes and notables. Journal of Animal Science, 80:E22-E32.

Freitas VJF, Melo LM, 2010. In vitro embryo production in small ruminants. Revista Brasileira de Zootecnia, 39, 409-413.

Galli C, Crotti G, Notari C, et al., 2001. Embryo production by ovum pick up from live donors. Theriogenology, 55: 1341-1357.

Galli C, Duchi R, Crotti G, et al., 2003. Bovine embryo technologies. Theriogenology, 59:599–616.

Galli Cesare, Roberto Duchi, Silvia Colleoni, et al., 2014. "Ovum pick up, intracytoplasmic sperm injection and somatic cell nuclear transfer in cattle, buffalo and horses: from the research laboratory to clinical practice", Theriogenology, 2014

Garcia, D M, Nera, A, Topie, E, et al., 2016. Chapter 2 In Vitro Fertilization (IVF) Obtaining Immature Oocytes via Ovum Pick Up (OPU).

Gardner DK, 1994. Mammalian embryo culture in the absence of serum or somatic cell support. Cell Bio l.Int., 116: 3-79.

Garner DL, Gledhill BL, Pinkel D, et al., 1983. Quantification of the X and Y chromosome-bearing spermatozoa of domestic animals by flow cytometry. Biology of Reproduction, 28: 312-321.

Ginther O J, Wiltbank MC, Fricke PM, et al., 1996. "Selection of the dominant follicle in cattle." Biology Reproduction, 55: 1187-1194.

Gonsalves PBD, Visintin JA, Oliveira MAL, 2002. In vitro embryo production. In: Gonsalves PBD, Figueiredo JR, Freitas VJF, editors. Biotechnologies Applied to Animal Reproduction. Varela, Sa˜o Paulo, SP; 2002. p. 195–226.

Gonzales-Bulnes A, Baird DT, Campbell BT, et al., 2004. Multiple factors affecting the efficiency of multiple ovulation and embryo transfer sheep and goat. Reprod Fert. Develop. J. 421 – 425.

Grondahl C, 2008. Oocyte maturation: Basic and clinical aspects of in vitro maturation (IVM) with special emphasis of the role of FF-MAS. Dan. Med. Bull., 55(1): 1-16.

Gümen A, Wiltbank MC, 2005. Length of progesterone exposure needed to resolve large follicle anovular condition in dairy cows. Theriogenology. 2005 Jan 1;63(1):202-18. doi: 10.1016/j.theriogenology.2004.04.009. PMID: 15589285

Hae-Lee Lee, Sue-Hee Kim, Dong-Beom Ji, et al., 2009. "A comparative study of Sephadex, glass wool and Percoll separation techniques on sperm quality and IVF results for cryopreserved bovine semen", Journal of Veterinary Science, 2009

Hammami, Sondes, 2014. Universitat Autònoma de Barcelona. Departament de Ciència Animal i dels Aliments. "In vitro embryo production from prepubertal goat oocytes in different culture media", [Barcelona] : Universitat Autònoma de Barcelona,, 2014

Hanuman Prasad Yadav, Sangram K Sahu , Shabir A Lone , et al., 2018. Advances in sperm sexing in bovines. J. Exp. Zool. India Vol. 21, No. 1, pp. 1-9

Hasler JF, 2003. The current status and future of commercial embryo transfer in cattle. Anim. Reprod. Sci., 79 (3-4), 245-264.

Hasler John F, Jennifer P. Barfield, 2015. In Vitro Fertilization DOI: 10.1002/9781118833971.ch81

Hendriksen PJM, Vos PLAM, Steenweg WNM, et al., 2000. Bovine follicular development and its effect on the in vitro competence of oocytes. Theriogenology, 53: 11-20.

Henkel, R R, & Schill, W, 2003. Endocrinology Sperm preparation for ART. (December). https://doi.org/10.1186/1477-7827-1-108

Herr Charles M, Klaus I Matthaei, Mark P Bradley et al., 1990. Rapid accurate sexing of livestock embryos. http://www.wcgalp.org/system/files/proceedings/1990/rapid-accurate-sexing-livestock-embryos.pdf

Herry Sonjaya, Hasbi Hasbi, 2019. "Potential of embrio production techniques for improving bali cattle seedstock ", IOP Conference Series: Earth and Environmental Science, 2019

Hyttel P, Fair T, Callesen H et al., 1997. Oocyte growth, capacitation and final maturation in cattle. Theriogenology 47, 23–32

Imai K, Tacawa K, Yoshioka H, et al., 2006. Efficiency embryo production by ovum pick up and in vitro fertilization in cattle. Journal of Reproduction and Development, 52:19-29.

Jafar SI, APF. Flint, 1996. "Sex selection in mammals: A review", Theriogenology, 1996

Jemal Jeilu, Tamrat Degefa, Tefera Yilma, et al., 2020. Ovarian follicular dynamics in Boran and Crossbred heifers in Ethiopia: Implications for assisted reproductive techniques. Ethiop. Vet. J., 2020, 24 (1), 54-72. DOI https://dx.doi.org/10.4314/evj.v24i2.

Kastelic JP, Knopf L, Ginther OJ, 1990a. Effect of day of prostaglandin F2 alpha treatment on selection and development of the ovulatory follicle in heifers. Animal Reproduction Science, 23: 169-180.

Kharche SD, AK Goel, SK Jindal, et al., 2006. "In vitro maturation of caprine oocytes in different concentrations of estrous goat serum", Small Ruminant Research, 2006

Kidder GM. and Vanderhyden BC, 2010. Bidirectional communication between oocytes and follicle cells: ensuring oocyte developmental competence. Canadian Journal of Physiology Pharmacology, 88:399–413.

Koopman, Peter, John Gubbay, Nigel Vivian, et al., 1991. "Male Development of Chromosomally Female Mice Transgenic for Sry gene." Nature 351 (1991): 117–21.

Lambert R, Sirard M, Bernard C, et al., 1986. In vitro fertilization of bovine oocytes matured in vitro and collected at laparoscopy. Theriogenology, 25:117-133.

Lane EA, EJ Austin, MA Crowe, 2008. "Oestrous synchronisation in cattle—Current options following the EU regulations restricting use of oestrogenic compounds infood-producing animals: A review", Animal Reproduction Science, 2008

Leibfried-Rutledge ML, Critser ES, Parrish JJ, et al., 1996. In vitro maturation and fertilization of bovine oocytes. Theriogenology, 31(1):61-74. 116

Lessard C, Siqueira LG, D'Amours O, et al., 2011. Infertility in a beef bull due to a failure in the capacitation process. Theriogenology, 76:891-899.

Levy N, Kobayashi S, Roth Z, et al., 2006. Administration of prostaglandin F2α during the early bovine luteal phase does not alter the expression of ET-1 and of its type A receptor: a possible cause for corpus luteum refractoriness. Biology of Reproduction, 3:377–382.
Lonergan P and Fair T, 2008. In vitro produced bovine embryos: Dealing with the warts. Theriogenology, 69: 17-22.
Lonergan P and Fair T, 2016 Maturation of Oocytes in Vitro. Annu. Rev. Anim. Biosci.,4: 255-268.
Lonergan P, 2003. "Oocyte and Embryo Quality: Effect of Origin, Culture Conditions and Gene Expression Patterns", Reproduction in Domestic Animals, 8/2003
Lonergan P, 2007. State of the art embryo technologies in cattle. Reproduction in Domestic Ruminants, VI. Nottingham University Press. Nottingham, UK. Pp. 315-25.
Machado, GM, 2009. "Effect of Percoll volume, duration and force of centrifugation, on in vitroproduction and sex ratio of bovine embryos", Theriogenology, 200905
Machaty, Z, J Peippo, and A Peter, 2012. "Production and manipulation of bovine embryos: Techniques and terminology", Theriogenology, 2012.
Malvezzi, H, Sharma, R, Agarwal, A, et al., 2014. Sperm quality after density gradient centrifugation with three commercially available media : a controlled trial. 1–7.
Manual on Assisted Reproduction, Springer Science and Business Media LLC, 2000
Mart ́ınez, MF, Colazo, MG, Kastelic, et al., 2003. Effects of estradiol and progesterone on plasma steroid and gonadotropin concentrations in CIDR-treated ovariectomized cows. Theriogenology, 59: 224, Abstr.
Martínez MF, Kastelic JP, Bo GA, et al., 2005. Effects of oestradiol and some of its esters on gonadotropin release and ovarian follicular dynamics in CIDR-treated beef cattle. Animal Reproduction Science, 86: 37–52. 29
Massip A, 1995. "Survival and viability of fresh and frozen-thawed in vitro bovine blastocysts", Reproduction Nutrition Development, 1995
Massip A, 2001. Cryopreservation of Embryos of Farm Animals. https://doi.org/10.1046/j.1439-0531.2001.00248.x
Miriam P. Cervantes, J. Manuel Palomino, et al., 2016. "In vivo and in vitro maturation of oocytes collected from superstimulated wood bison (Bison bison athabascae) during the anovulatory and ovulatory seasons", Animal Reproduction Science, 2016
Moreira FR, de la Sota L, Diaz T, et al., 2000. Effect of day of the estrous cycle at the initiation of a timed artificial insemination protocol on reproductive responses in dairy heifers. Journal of Animal Science, 78: 15681576. https://doi.org/10.2527/2000.7861568x
Mufeed A. Alnimer, 2005. "Comparison of an oestrus synchronisation protocol with oestradiol benzoate and PGF2Î± and insemination at detected oestrus to a timed insemination protocol (Ovsynch) on reproductive performance of lactating dairy cows", Reproduction Nutrition Development, 11/2005
Naitana S, S Ledda, 2020. "Reproductive technologies in sheep", Elsevier BV, 2020
Natumanya, R, Owiny, D, & Kugonza, DR, 2008. The Potential of Ankole Cattle Abattoir Ovaries for In Vitro Embryo Production. AJABS Vol 3 (1), 2008.
Oliveira LZ, de Lima VFMH, Levenhagen MA, et al., 2011. Transmission electron microscopy for characterization of acrosomal damage aier Percoll gradient centrifugation of cryopreserved bovine spermatozoa. Journal of Veterinary Science,12: 267-272
Parrish JJ, 2014. Bovine In vitro fertilization: In vitro oocyte maturation and sperm capacitation with heparin. Theriogenology, Author manuscript; available in PMC 81(1): doi:10.1016/j.theriogenology.

Parrish JJ, Susko-Parrish JL, Uguz C, et al., 1994. Differences in the role of cAMP during capacitation of bovine sperm by heparin or oviduct fluid. Biol. Reprod., 51:1099-108.

Parrish JJ., Krogenaes A and Susko-Parrish JL, 1995. Effect of bovine sperm separation by swim-up or percoll on success of in vitro fertilization and embryo development. Theriogenology, 44:859-69.

Paul Ashit Kumar, Ton Yoisungnern and Napolean Bunaparte, 2015. Hormonal treatment and estrus synchronization in cows: A mini-review J. Adv. Vet. Anim. Res., 2(1): 10-17 ,March 2015. DOI: 10.5455/javar.2015.b45

Perez O, Richard IR, Green HL, et al., 2000. Ultrasound guided transvaginal oocyte recovery from FSH-treated post-partum beef cows. Theriogenology, 53: 364.

Pieterse MC, Kappen KA, Kruip TAM et al., 1988. Aspiration of bovine oocytes during transvaginal ultrasound scanning of the ovaries. Theriogenology, 30: 751-761.

Prentice-Biensch JR., Singh J, Alfoteisy B, et al., 2012. A simple and high-throughput method to assess maturation status of bovine oocytes: comparison of anti-lamin A/C-DAPI with an aceto-orcein staining technique. Theriogenology, 78:1633–1638.

Pursley JR, Mee MO, Wiltbank MC, 1995. Synchronization of ovulation in dairy cows using PGF2α and GnRH. Theriogenology, 44:915–23.https://doi.org/10.1016/0093691X(95)00279-H

Répási A, Beckers J F, Sulon J, et al., 2005. Effect of the type and number of prostaglandin treatments on corpus luteum, the largest follicle and pro-gesterone concentration in dairy cow. Reproduction in Domestic Animals, 40: 1–7.

Rezazadeh Valojerdi M, Eftekhari-Yazdi P, Karimian L, Hassani F, et al., 2009. Vitrification versus slow freezing gives excellent survival, post warming embryo morphology and pregnancy outcomes for human cleaved embryos. J Assist Reprod Genet. 2009; 26(6):347-354. doi:10.1007/s10815-009-9318-6

Rivera H, Lopez H, Fricke PM, 2004. Fertility of Holstein dairy heifers after synchronization of ovulation and timed AI or AI after removed tail chalk. Journal of Dairy Science, 87: 2051–2061.

Rizos D, Gutierrez-Adan A, Perez-Garnelo S, et al., 2003. Bovine embryo culture in the presence or absence of serum: implications for blastocyst development, cryotolerance, and messenger RNA expression. Biol Reprod, 68:236-243.

Rizos D, Ward F, Duffy P, et al., 2002. Consequences of bovine oocyte maturation, fertilization or early embryo development in vitro versus in vivo: implications for blastocyst yield and blastocyst quality. Molecular reproduction and development, 61:234-48.

Rodgers JC, Bird SL, Larson JE, et al., 2015. An economic evaluation of estrous synchronization and timed artificial insemination in suckled beef cows. Journal of Animal Science, 10: 1297-1308. 6

Rodríguez Villamil P, Wei H, Moreira G, et al., 2012. Fertilization rates and in vitro embryo production using sexed or non-sexed semen selected with a silane-coated silica colloid or Percoll. Theriogenology. Jul 1;78(1):165-71. doi: 10.1016/j.theriogenology.2012.01.033. Epub 2012 Apr 10. PMID: 22494675.

Ryan M, Mihm M, Roche JF, 1998. Effect of GnRH given before or after dominance on gonadotrophin response and the fate of that follicle wave in postpartum dairy cows. Reproduction and Fertility, 57: 61-71.

Saini N, M K Singh, S M Shah, et al., 2015. "Developmental competence of different quality bovine oocytes retrieved through ovum pick-up following maturation and fertilization ", Animal, 2015

Samardzija M, Getz I, Lojkic M, et al., 2015. Optimization of Sperm for In vitro Production of Bovine Embryos. SOJ Vet Sci 1(2): 1-7. DOI: http://dx.doi.org/10.15226/2381-2907/1/2/00107

Samardžija M, Karadjole M, Cergolj M, et al., 2005. The comparison of the two bull sperm separation methods for in vitro fertilization. Tierarztl. Umschau,60 (4):193-199

Santos JEP, Thatcher WW, Chebel RC, et al., 2004. The effect of embryonic death rates in cattle on the efficacy of estrus synchronization programs. Animal Reproduction Science, 82:513-535.

Sarkar M, BK Dutta Borah, BS Prakash, 2009. "Strategies to optimize reproductive efficiency by regulation of ovarian function in yak (Poephagus grunniens L.)", Animal Reproduction Science,

Seidel GE, Schenk JL, 2008. Pregnancy rates in cattle with cryopreserved sexed sperm: effects of sperm numbers per inseminate and site of sperm deposition. Anim Reprod Sci, 105:129-138

Seidel JGE, 2012. Sexing mammalian sperm; where do we go from here? Journal of Reproduction and Development, 58: 505-509.

Seyoum1 and Lemma, 2022. Breeding soundness of semen producing bulls, their semen quality and inter relationship of functional tests with spermatozoa motility percentage. J. Vet. Med. Anim. Health PP 1-11

Sharma Mridula, Anoop Singh, Nishant Sharma et al., 2019. Embro sexing in cattle: Review open access at journalijcir.com

Shirazia A, Shams-Esfandabadia N, Hosseinia SM, et al., 2007. The presence of cumulus cells on nuclear maturation of sheep oocytes during in vitro maturation. Small Rumin Research, 68:291–295.

Singh B, 2009. "Reproductive biotechniques in buffaloes (Bubalus bubalis): status, prospects and challenges", Reproduction Fertility and Development, 2009

Stevenson JS, Johnson SK, Milliken GA, 2003. Symposium Paper: Incidence of postpartum anestrus in suckled beef cattle: Treatments to induce estrus, ovulation, and conception. Professional Animal Science, 19:124–134

Stevenson JS, Tenhouse DE, Krisher RL, et al., 2007. Detection of anovulation by heat mount detectors and transrectal ultrasonography before treatment with progesterone in atimed insemination protocol. Journal of Dairy Science, 91: 2901–2915.doi:10.3168/JDS.2007-0856

Sudano MJ, Paschoal DM, Rascado TD, et al., 2011. Lipid content and apoptosis of in vitro-produced bovine embryos as determinants of susceptibility to vitrification. Theriogenology, 75:1211-1220.

Thatcher WW, Bilby TR, Bartolome JA, et al., 2006. Strategies for improving fertility in the modern dairy cow. Theriogenology, 65:30-44

Van Wagtendonk-de Leeuw AM, 2006. Ovum pick-up and in vitro production in the bovine after use in several generations: a 2005 status. Theriogenology, 65(5): 914-25.

Vasconcelos JL, Silcox RW, Rosa GJ, et al., 1999. Synchronisation rate, size of ovulatory follicle, and pregnancy rate after synchronization of ovulation beginning on different days of the estrouscycle in lactating dairy cows. Theriogenology, 52: 1067-1078.

Velez I C, C Arnold, C C Jacobson, et al., 2012. Effects of repeated transvaginal aspiration of immature follicles on mare health and ovarian status. Equine Veterinary Journal. https://doi.org/10.1111/j.2042-3306.2012.00606.x

Verma OP, Kumar R, Kumar A, et al., 2012. Assisted reproductive techniques in farm animal - From artificial insemination to nanobiotechnology. Vet. World. 2012;5(5):301–310.

Viana JHM, 2017. Statistics of embryo collection and transfer in domestic farm animals Embryo Transfer. Newsletter, 36: 08-25.
Viana JHM, Ferreira AM, Sá WF et al., 2000. Follicular dynamics in zebu cattle. Pesquisa Agropecuária Brasileira, 35: 2501-9.
Viana JHM, Figueiredo ACV, Gonçalves RLR et al., 2018. A historical perspective of embryo-related technologies in South America. Animal Reproduction, 15: 963-970.
Vishwanath R, 2003. Artificial insemination: the state of the art. Theriogenology, 59: 571-584.
Ward F, Enright B, Boland M, et al., 2002. Optimization of in vitro bovine embryo production: effect of duration of maturation, length of gamete co-incubation, sperm concentration and sire. Thriogenology, 57: 2105-2117.
Xiao-Yu Yang, Hua Li, Wen-Ying Huang, et al., 2007. "Comparison of Two Different Schemes of Once-weekly Ovum Pick Up in Dairy Heifers", Asian-Australasian Journal of Animal Sciences, 2007
Yang X-Y, P Chen, Y-F Huang, et al., 2008. "The timing of first cleavage of different cloned and IVF embryos with oocytes recovered by ovum pickup", Journal of Zoology, 2008
Yavetz H, Hauser R, Homonnai ZT, et al., 2018. Separation of sperm cells by sedimentation technique is not suitable for in vitro fertilization purposes. Andrologia. 2018; 28: 3–6. https://doi.org/10.1111/j.1439-0272.1996.tb02750.x PMID: 8659712
Youngs CR, SP Leibo, and RA Godke, 2010. Embryo cryopreservation in domestic mammalian livestock species doi: 10.1079/PAVSNNR20105060