Available online at www.worldscientificnews.com

World Scientific News

An International Scientific Journal

WSN 157 (2021) 169-188 EISSN 2392-2192

Advances in Bovine Follicular Aspiration Technique

Sayid Ali

Debre Zeit Agricultural Research Center, Ethiopian Institute of Agricultural Research, Bishotu, P.O. Box: 32, Ethiopia

E-mail address: sayid1731@gmail.com

ABSTRACT

The ability to produce large number of embryo from donor of high genetic merit using assisted reproductive technology has a considerable potential value. It is vital in disseminating genetic improvement, shortening the progeny testing and generation interval. Live animal and slaughter house ovary are good source of oocytes for in vitro embryo production. Oocytes are recovered from ovaries of slaughtered animals by using needle aspiration, slicing, aspiration and dissection or dissection alone as a method of recovering oocytes. *In vitro* embryo production from live donors through laparoscopic methods of oocyte recovery or transvaginal oocyte recoveries are extremely versatile techniques because they can be applied to donors of all ages from two-month-old calves to very old cows. It can be used for dry and lactating donors and even during pregnancy up to the third or fourth month. It does not interfere with the physiological status of the donor since no hormonal stimulation is required. However, in certain instances, it can have a beneficial effect. It is normally applied in a regime of two ultrasound-guided oocyte collections per week. Thus in a very short time several batches of embryos fertilized with the desired bulls can be obtained. Virtually all female cattle starting from two months of age can be oocyte donors by follicular aspiration. The only exceptions are pregnant animals after the third or fourth month of pregnancy and animals with severe ovarian hypoplasia or in the immediate post-partum before Ovarian activities are restored. Several factors such as breed, age, oocyte quality, reproductive status body condition follicular environment, fertilization, and embryo culture environment affect oocyte recovery rate, quality and quantity of the oocyte.

***Keywords*:** Bovine Oocyte retrieval, Aspiration, Dissection, Slicing, OPU, LOPU, Factors

(Received 16 April 2021; Accepted 07 May 2021; Date of Publication 08 May 2021)

1. INTRODUCTION

The world food crisis on one hand and the rise in demand for food of animal origin, on the other hands plus the negative effects of climatic change, especially in the tropical areas urgently demand innovative strategies and interventions to be employed. Accordingly, many attempts aimed at improving the productivity of indigenous dairy cattle in developing countries through crossbreeding with temperate breeds. The development of biotechnologies in the fields of breeding, reproduction and molecular genetics has advanced considerably in recent years and become the most emblematic products of finalized research in the domain of livestock development, globalization of genetic exchange and conservation.

Assisted reproductive technologies like Artificial insemination (AI), embryo transfer (ET), *in vitro* embryo production (IVEP) and others allow producers to make continued genetic advancements – speed up genetic progress, improve performance, reduce the risk of disease transmission, and expand the number of animals that can be bred from the superior parent. It also helps in regulations of import and export of genetic materials, characterization and conservation of species threatened by extinction. In most domestic animals including sheep and goats, the genetic gain is limited on the female side simply due to the naturally determined reproductive rate rather than the other factors. Embryo transfer technique is an outstanding technique that can be used to produce remarkable number of progenies. This results in rapid genetic gain, which complements the AI program as it involves the use of superior female as a donor of embryo. There are two ways of embryo production technology: (i) multiple ovulation and embryo transfer (MOET), and (ii) *in vitro* embryo production through *in vivo* Ovum pick up (OPU), and oocyte recovery from slaughter.

Normal newborn female calf has enormous potential in reproducing in terms of all the oocytes stored in her ovaries. For example, a beef heifer may have 150,000 or more primordial follicles containing oocytes in their ovaries at the time of birth. Nevertheless, as female cattle might only produce on average 10 calves in her lifetime, what happens to the remaining 149,990 oocytes of the pool? Usually, non-pregnant cyclic cows ovulate at 20- to 22-day intervals removing one ovum from this oocyte pool each cycle. Furthermore, follicles in cattle develop in waves of 4 to 12 follicles at a time, and a cow usually has two to three waves of these growing follicles during a typical estrous cycle. And hence, 12 to 36 follicles develop to varying degrees and most of them regress during this interval, with usually only one follicle ovulating in a given cycle. Follicular waves have also been reported to occur during the early part of pregnancy, again removing oocytes from the pool. This difference in female gamete production presents a unique paradox for discussion; however, the answer the producers are most often seeking is how can they maximize utilization of the gametes from their favorite females?

Over the years, MOET technology has been used to increase the number of possible offspring from selected females, however, there are still drawbacks associated with this approach. For example, some females do not respond to the stimulatory agents, hormone-treated donors also develop cystic follicles during the process or develop physiological conditions that make it difficult retrieving the embryos.

IVEP, the procedure involving harvesting the oocytes from the cow's ovaries and fertilizing them *in vitro*, is an alternative technology that overcomes the drawbacks arising from conventional MOET. The oocyte for IVEP can be harvested from slaughterhouse ovaries (from voluntary and involuntary culls) as well as from live donor cow. Slaughterhouse derived ovaries come from cattle with diverse reproductive background, and contain a highly heterogeneous population of oocyte and developmental competence that can be harvested either by aspiration and dissection. Immature oocytes can be collected from the ovaries of life animal by the minimally invasive laparoscopic ovum pick up, LOPU method or by follicular aspiration. Therefore, the objectives of this review are:

- to review the oocyte retrieval technique from different types of donors, including juvenile and pregnant animals
- to review different factors affecting oocyte recovery, quality and developmental competency of the recovered oocyte.

2. CURRENT STATUS OF IN VITRO EMBRYO PRODUCTION IN THE WORLD

2. 1. Embryo collection and transfer in domestic farm animals

The transfer headcount of the IVP embryos increased year by year. In fact, when compared with conventional MOET, the IVEP can produce a greater number of embryos in a limited period because IVEP uses oocytes that can be obtained from different types of animal including pregnant donors, donors with reproductive disorder and pre-pubertal calves with optimal oocyte retrieval technique like OPU in live animal and from slaughterhouse ovaries with low cost. It was 390 in 1987, exceeded 9,000 in 1997, increased to be 11,653 in 2000, and again it reached 13,204 heads in 2007. The conception rates of frozen and fresh embryos were 39% and 40%, respectively in 2012. The conception rate of fresh IVP embryo was maintained more than 40% from 2001 to 2012, and that of frozen IVF embryo gradually increased from 1997 to 2012.

In cattle, 1,487,343 transferrable embryos were collected or produced in 2017, from which 33.3% (495,054) were *in vivo*-derived (IVD) and 66.7% (992,289) were *in vitro*-produced (IVP) embryos. Compared to 2016, there was a 48.9% increase in the number of IVP embryos recorded, whereas the number of IVD embryos collected decreased by 21.7%, resulting in a 2-fold difference between IVP and IVD totals (992,289 vs. 495,054, respectively).

The main factor driving this change was a remarkable growth of IVEP both in North America (+82.7%) and Europe (+164.7%). The development of IVEP in these regions affected the world ET scenario and, for the first time since 1999, the number of IVP embryos in North America was greater than in South America. This accounts for transvaginal ultrasound-guided follicular aspiration (OPU) that remains the main source of oocytes for IVEP.

3. OOCYTE QUALITY AND GRADING PARAMETER

3. 1. Oocyte quality and grading system

The Ovaries are the primary organs in a bovine's reproductive tract which produce egg. Ovaries also produce estrogen and progesterone hormone throughout the different stage of the estrous cycle. Follicle and corpus luteum can be found on the surface of the ovary. Follicles are fluid filled, blister like structure which contain developing oocyte. Normally there are follicles with variation in size from barely visible to 18 to 20 mm in diameter found on each ovary. The largest follicle present is the growing follicle and is used for ovulation when the bovine comes in to heat. Over time, more than 95% of the other follicles on the ovary egress and die without ovulating and are replaced by new growing follicles.

Oocyte is an immature female sex unit which starts as an oogonium by the process of oocytogenesis, and matures to become fully mature egg cell or ovum. Oocytes are female germ cells involved in reproduction and these are the largest cells (almost 0.12-0.50 mm in diameter) of mammalian body, which are typically visible to naked eyes without use of the magnification device. Oocytes in cattle are formed during embryogenesis of female fetus and develop within individual follicles in the cortex of the ovary.

The bovine reproductive egg, the oocyte, is collected in the form of a cumulus oocyte complex (COC) during aspiration of the follicles for IVEP. The COC is the oocyte surrounded by the bank of cells (cumulus cells), inside which it sits in the ovary (**Figure 1**). The quality and developmental stage of the COC collected influences the maturation of the oocyte, its fertilization efficiency and subsequent development of the embryo, as well as the pregnancy rates of the subsequent transferred embryo.

Figure 1. Oocyte surrounded by the bank of cells

3. 2. Oocyte classification schemes

There are various systems used in the grading of bovine oocytes. Mostly it depends on a visual subjective appraisal by laboratory personnel and can vary with the individual one and the laboratory. Selection of bovine oocytes for IVM and IVEP on the basis of visual assessment of morphological features has been examined by several groups and there have been many reports describing classification schemes.

Retrieved Oocytes could be examined under stereomicroscopy and classified into four categories according to their compaction, number of cumulus cell layers and homogeneity of ooplasm various constituents of follicular fluid and their concentration may also be useful in assessing the quality of the oocyte:

- **Class A)** Cumulus oocytes–complexes (COCs) with compact multi-layers cumulus cells (≥3 layers) and homogeneous ooplasm, total COC light and transparent
- **Class B)** Compacted multi-layer cumulus investment homogeneous ooplasm but with a course appearance and dark zone at the periphery of oocyte, total COC, slight darker and less transparent
- **Class C)** Less compacted cumulus investment ooplasm irregular with dark cluster, total COC darker than A and B

- **Class D)** Expanded cumulus investment cumulus cell scattered in dark clumps in a gelly matrix ooplasm irregular with dark cluster, total COC, dark and irregular (**Figure 2**).

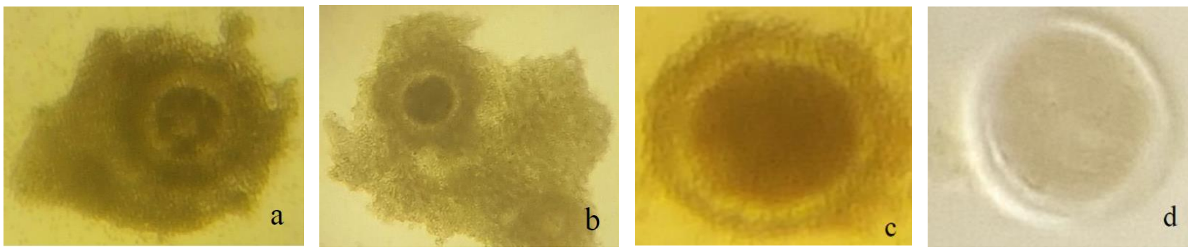

Figure 2. Different grades of bovine Oocytes

A thick layer of cumulus cells may provide an efficient level of nutrients and other factors to the oocyte in the stages of development. Therefore, during recovery of oocytes with a proper technique, grading of the COCs is the first and very important step in the IVEP of oocytes in respect to quality and quantity.

4. SOURCE AND RETRIEVAL TECHNIQUES OF OOCYTE

The number of good quality oocytes harvested from the ovary is an important consideration in the IVEP. Oocytes for IVF are collected from one of the following sources: the oviducts – soon after ovulation, mature follicles shortly before ovulation or from immature and atretic follicles from abattoir derived material.

Various techniques have been used to collect oocytes aseptically from the bovine ovaries. The recovery technique will vary according to whether pre- or post-ovulatory oocytes are required and whether collection is to be made from slaughtered animals at the slaughter house or from live cattle on single or repeated occasions. Procedurally, oocytes can be recovered from ovaries of slaughtered cows by using aspiration, slicing or puncturing as methods of recovering oocytes whereas oocytes can be recovered from live cows using the ultrasound guided transvaginal oocytes retrieval-ovum pick up. This recovery permits the production of more embryos than might be possible by the standard ET practice following IVEP. The oocyte yield and quality vary with the harvesting technique employed and depends on the reproductive status of the donor, season, species and breed.

4. 1. Aspiration technique for collection of Oocyte from slaughterhouse ovaries

Large numbers of embryos can be produced from the ovaries of slaughtered donors when the female parental origin is not required to be known. In this case, the ovaries from donors of the same breed are pooled and processed as a batch. This procedure significantly simplifies all the steps involved in production, identification and freezing and, as a consequence, embryos are produced at low cost and can be commercialized at very competitive pricing. This type of production is used for premium beef breeds for the commercial production of beef calves.

When a donor of high genetic value is slaughtered for various reasons (terminal illness, infertility, age, etc.) the ovaries can be collected, processed separately from those of other donors and the oocytes matured and fertilized with the sire required by the client. This procedure, often defined "genetic recovery", allows the production embryos of known parentage. The expected results in terms of embryo production are related to the reasons for the slaughter. The outcome is usually poor (1-2 embryos per donor) for what we call "terminal" donors. This definition includes animals that are already dead or are in critical general conditions, such as immediate post calving problems, acute mastitis, displaced abomasum, progressed foot or leg injuries, etc. In the case of healthy donors that are slaughtered because of infertility, end-of career or for the eradication of infectious diseases, the outcome is much better with an average production of 6 or more embryos per donor.

Commonly there are several techniques used to collect oocyte from the bovine ovaries at slaughterhouse (abattoirs) which are: dissection, aspiration, slicing and aspiration plus slicing technique. Recovery of bovine oocytes by aspiration of antral follicles, using various devices (pipette, syringe and needle, aspiration needle under vacuum pressure), has been the method most commonly employed with cattle ovaries retrieved from the slaughterhouse. The oocytes were aspirated from individual ovaries after carefully removing the extraneous tissues and placed in Petri dish containing 1 mL of PBS. Oocytes were aspirated from the visible follicles presented on the ovarian surface (**Figure 3**) with 22 gauge needle fixed to 5 mL disposable syringe containing 1~2 mL of PBS. Aspiration technique is one of the selected techniques of oocyte recovery from the slaughterhouse because of its speed which may be particularly important in a commercial embryo production unit of operation as opined.

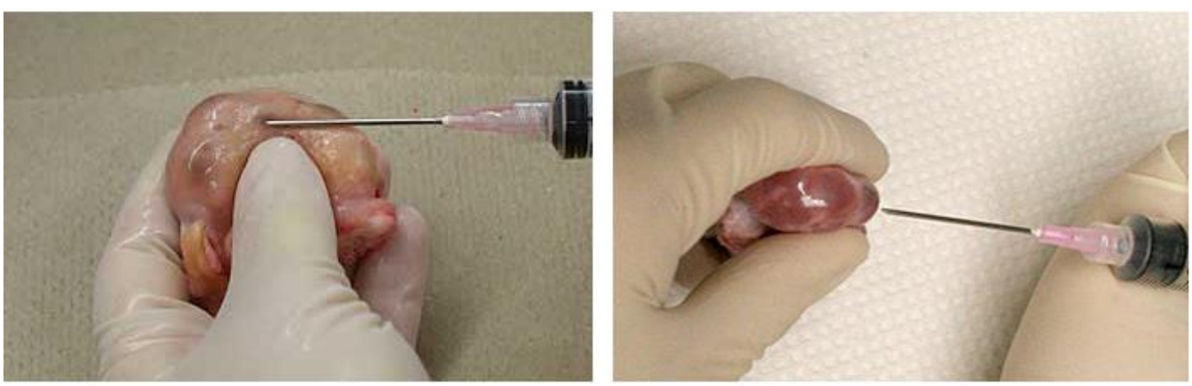

Figure 3. Aspiration method for Oocytes collection.

Moreover, it is also the most commonly employed technique in retrieving bovine COC's from slaughterhouse ovaries. It is advantageous because it yields more intact and unexpanded cumulus oocyte complexes from the visible follicles of 2-8 mm diameter, together with a higher proportion of culture grade oocytes with less ovarian tissue debris, and requires less repeated washing that would otherwise result in denudation of the surrounding cumulus cells. One of the difficulties by the aspiration method is that only 30-60% oocytes may be retrieved from the punctured follicles. This could contrast markedly with follicle dissection where 100% recovery was not unusual. Quality-wise, it was common experience to find this to be lower with aspiration than with follicle dissection.

4. 1. 1. Ovary slicing techniques

Several studies have reported on oocyte recovery techniques involving the slicing, cutting or dissection of the bovine ovary as a means of recovering immature oocytes. Slicing techniques have been either directly applied to ovaries or applied after completion of aspiration. Ovarian samples after detached smoothly and firmly from surrounding tissues and fat were washed with disinfectant water and normal saline then suspended in beaker containing medium with antifungal and antibiotic preparations at room temperature. As indicated in **Figure 4**, the ovaries were held firmly with the help of forceps in a sterile glass Petri dish and then sliced into possible thin sections with a surgical blade.

Some workers have reported a threefold increase in oocyte numbers compared with aspiration while others have shown the method to be capable of acquiring greater oocyte numbers in sheep and goat ovaries. Dublin workers found that slicing took about three times longer per ovary than aspiration, but this was compensated by the threefold increase in yield. In terms of workload, slicing provided the same number of oocytes per unit of time as aspiration, but with many fewer ovaries. The number of oocytes recovered and the number of blastocysts obtained was approximately doubled by slicing ovaries in comparison with aspiration. This is due to the fact that those located in follicles under the ovarian surface (cortical location) are obtained in addition to those located in peripheral (surface visible) follicles. When the numbers of oocytes from both peripheral and cortical follicles are combined, it is apparent that the total yield is about double that collected from one ovarian site alone. Apart from being time consuming, slicing is open to criticism on the grounds of risking contamination of the COCs during the collection procedure.

4. 1. 2. Aspiration plus slicing technique

The aspirated ovaries were subjected to further slicing to obtain count of the residual oocytes or post aspiration slicing (Figure 4).

Figure 4. Aspiration plus slicing method **(A)** & Slicing **(B)** of bovine Oocytes collection

Several reports have dealt with the slicing of ovaries after the preliminary aspiration of follicles; in terms of oocytes recovered or their quality, there appeared to be no merit in combining the two procedures. The effect of different collection techniques on oocyte recovery rate and, being the higher for aspiration plus slicing and slicing alone (84.7 and 83.3%) than for aspiration alone (72.7%), while dissection technique showed the lowest oocyte recovery rate in bovine (52.0%).

4. 1. 3. Ovarian slashing/Dissection technique

In dissection technique, individual follicles are dissected from the ovaries by using scissors and forceps (**Figure 5**) and the number can be recorded. Ovarian sample after detached smoothly and firmly from surrounding tissues and fat should be washed with distilled water and normal saline then suspended in beaker containing PBS with antifungal and antibiotic preparations at room temperature. Each one entire organ would be grasped or snatched well by artery forceps slightly over the solution preparation inside the beaker, by a fine surgical blade the snatched organ would be firmly clipped mainly over the follicles many times in which the follicular fluid would be dropped in the medium below with involving oocytes; then the whole entire organ was stripped and sheared transversally by the blade to be sure that all the content will be dropped down in the medium.

Figure 5. Dissection methods of oocytes collection

4. 2. Recovering Oocytes from live animal

4. 2. 1. Ultrasound guided Oocyte recovery (OPU)

In mid 1980s, the new reproductive technology OPU was developed to harvest oocytes from live cattle. This method was called ovum pick-up, and was established as the collection method of human oocytes to assist human infertility and applied to bovine. The technique of ultrasound-guided transvaginal follicular aspiration for OPU is a non-invasive procedure for recovering oocytes from antral follicles in live animals.

The most flexible and repeatable technique to produce embryos from any given live donor is offered by the technique of OPU or ultrasound guided follicular aspiration. A scanner with an adequate endovaginal (or adapted for the vaginal use) sector probe with a guided needle is required to perform this procedure. The needle is connected to a test tube and to a vacuum pump to aspirate the follicular fluid and the oocyte contained in it. A scanner with good resolution and with a probe of at least 6 MHz is used to envisage follicles down to 2-3 mm in size and also to view the needle during follicle aspiration (**Figure 6**). OPU has virtually no drawbacks for the donor and can even have a therapeutic effect in some infertile donors affected by ovarian cystic syndrome or similar pathologies that compromise reproductive function.

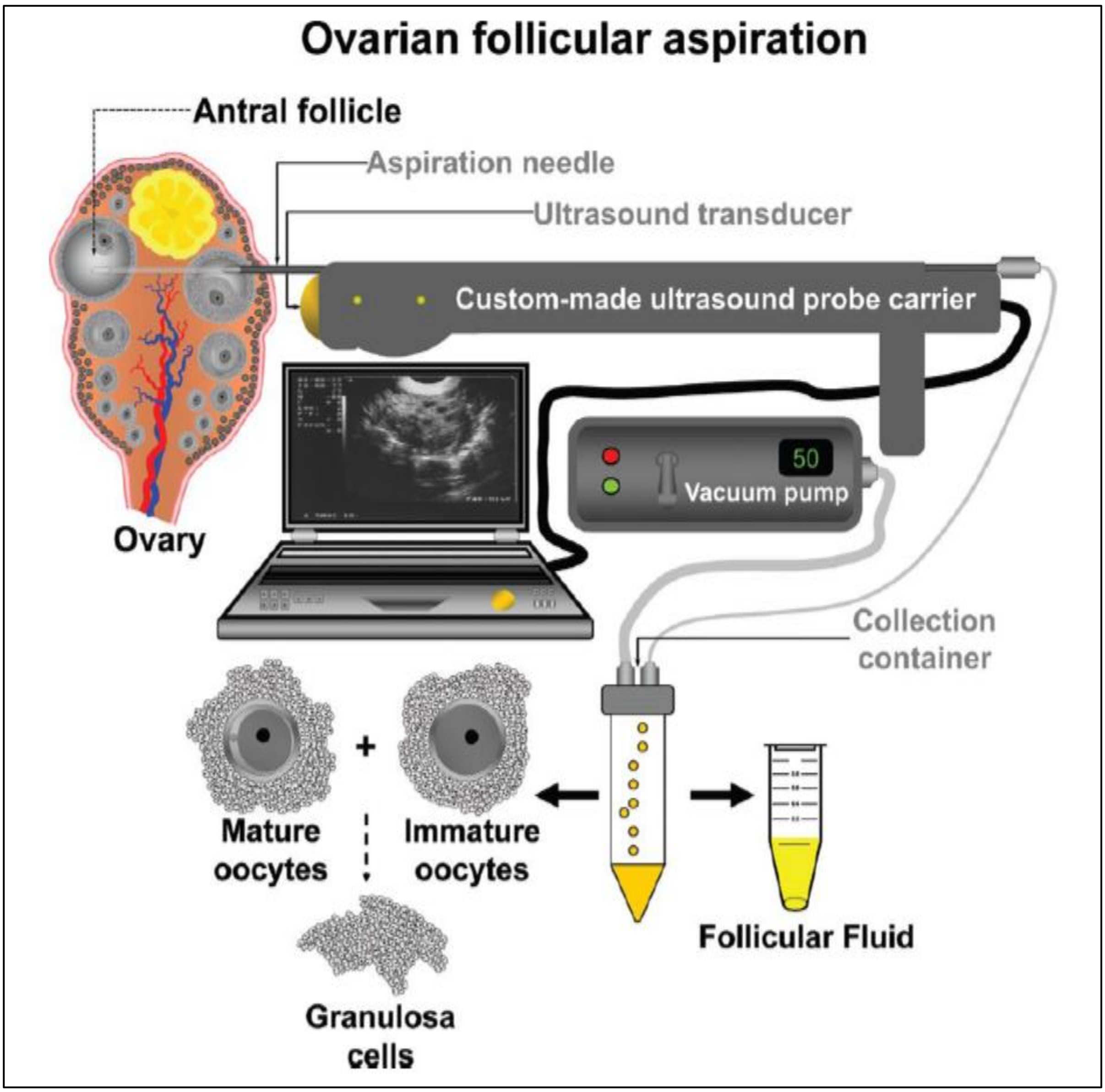

Figure 6. Schematic representation of the setup in transvaginal ultrasound guided follicular aspiration (OPU) in live cow.

Embryo production by conventional superovulation techniques may lead to fewer than 100 embryos over the lifetime of an individual cow, even though there may be 150,000 or up to half a million oocytes present in the ovaries. If more of these oocytes could be turned into embryos this is likely to have a considerable impact on cattle breeding programmers. *In vitro* embryo production from problematic, pregnant and acyclic cows by transvaginal OPU is being employed to an increasing extent around the world. *In vitro* embryo production is used almost exclusively to obtain young from problem cows that cannot be dealt with by conventional ET technology; the authors provided data showing the capability of such problem cows to produce embryos that resulted in pregnancies.

4. 2. 2. Advantages and alternatives of OPUs over the conventional superovulation

Compared with conventional superovulation and ET, the 3rd generation ART, production of embryos in the laboratory called IVEP technique came to picture by fulfilling advantages missed out with earlier generations. Some of the advantages include:

- It avoids the variability problem with the response to super-ovulation treatments in the context of *in vivo* collection of embryos. OPU can be performed sporadically or on a regular basis such as two times a week for many weeks or months.
- It enables to obtain a much higher number of oocytes per female than in the preceding technique since frequent repetition, twice a week protocol is the one that yields the maximum number of competent oocytes in a given period of time. Example, *via* OPU, a donor European or zebu cow may yield 15-20 oocytes each week (collection of 5-10 twice a week or 15-20 oocytes once a week, respectively). Considering the usual rates of development and losses obtained after IVEP and ET, a cow may potentially produce 50 to 100 calves each year. Virtually any female starting from six months of age up to the third month of pregnancy and also soon after calving (2-3 weeks), is a suitable donor. It can be applied successfully irrespective of the reproductive status of the donor, *i.e.* in pregnant, prepubertal females and acyclic animals, in those having patent tube (cows with blocked fallopian tubes) or genital tract infections and in animals non-responsive to superovulatory treatment. This makes OPU a very flexible technique that, unlike MOET, does not interfere with the normal reproduction and production cycles of the donor.
- In addition, to fertilize oocytes harvested from a cow with semen from different bulls (dam sire combinations) resulting in embryos with different sires being and increase the number of offsprings obtained by far than that of MOET from donors with high genetic value; example, via OPU, a donor European or zebu cow may yield 15-20 oocytes each week (collection of 5-10 twice a week or 15-20 oocytes once a week, respectively). Considering the usual rates of development and losses obtained after IVEP and ET, a cow may potentially produce 50 to 100 calves each year. IVEP shortens the generation interval, since female calves (2-3 months of age) have been used as oocyte donors by OPU conducted *via* laparotomy.

4. 2. 3. Different OPU System

The main process of OPU includes epidural anesthesia, ovary positioning per rectum, follicle visualizing by the transvaginal transducer, and oocyte aspiration by needle. There are two major OPU systems including non-stimulation and pre-stimulation procedure. As the name implies, the difference is whether the donor will be stimulated with hormone prior to OPU.

The original OPU procedure includes no hormone stimulation. It routinely performs twice a week, which allows the maximum recovery of oocytes of suitable quality for embryo production in a given time interval compared to once-a-week OPU, because no dominant follicle develops when all visible follicles are aspirated in the OPU process. While in most once-a-week collections, a dominant follicle develops at the successive collection, which causes the regression and degeneration of the subordinate follicles. In a result, oocytes collected by this scheme are relatively less in a given time interval. On a per cow per session basis, there was no difference between 'OPU 1/w' and 'OPU 2/w' protocols in terms of the average number of follicles aspirated, oocytes retrieved and blastocyst produced on Day 7, While on a weekly basis, those three indexes were significantly higher in the 'OPU 2/w' protocol than those in the 'OPU 1/w'. Moreover, as it does not interfere with the normal reproduction cycles of the donor, there were not any long-term detrimental effects on the donor cow's fertility even after twice-a-week OPU for over a year performed by experienced operators.

The main development in the OPU research is the hormone involved. The advantages of super stimulation prior to OPU seem obvious more follicles to puncture, more oocytes retrieved and more embryos and less work as compared to non-super stimulated OPU. In spite of all cited improvements above, the hormone-stimulated OPU has some unsolved key problems. Application of exogenous hormones disturbs donor's endocrine, especially when exogenous hormones are used to the donor for a long time, which might lead to infertility of the donor. Moreover, donor's responses to hormone stimulation are different, even the same donor shows different responses in different sessions, which leads to variation of the results. As a result, it is better to use hormones to the donor in a short period and leave a period of regulation and recovery to the endocrine system.

4. 3. Laparoscopic (LOPU) methods of follicular aspiration

Laparoscopic Ovum Pick-Up (LOPU) is the most reliable and efficient technique for collecting high quality oocytes from live animals in certain species or age groups, allowing its use in IVEP. The technique is minimally invasive and has repeatability and reliability, allowing the observation of the ovaries through the laparoscope and the puncture of the follicles. In contrast to MOET, LOPU brings the advantages of being less invasive and causing less postoperative stress, and can be repeated in a shorter interval (minimum of 60 days for MOET against a minimum of 14 days for LOPU).

4. 3. 1. Description of the LOPU Technique

The equipment used for LOPU consists of a laparoscope (diameter of 5 or 7 mm, angle of 0º or 30º and length of ~ 30 cm) connected to a cable and light source (**Figure 7**). One pump of insufflation, three trocars (two of 5 mm being one with valve for insufflation and one of 3.5 mm) and atraumatic endoscopic forceps. The technology has been applied to species or age groups where it is not possible or easy to manipulate the reproductive tract through the rectum during oocyte retrieval.There are two big categories of applications for the LOPU-IVEP technology in production animals, those in which it acts as an alternative to MOET (competitive applications) and those in which it doesn't compete with MOET, as MOET cannot be done in those categories (noncompetitive applications).

4. 3. 2. Competitive Applications

This application refers to the use of non-gravid, non-lactating/weaned, adult females as donor of oocytes for LOPU-IVEP for the exact same objective as MOET, *i.e.* production of more progeny from outstanding adult females. That in a 1:1 comparison, MOET should win this competition because in normal conditions MOET should result in an average of 8-10 transferable embryos/flush while LOPU-IVEP produces an average of 4-5 blastocysts for transfer, and the viability of *in vivo* produced embryo tends to be better than the *in vitro* counterparts. So, in this specific application, the advantage of using LOPU-IVEP applies only to customers willing to produce many more progenies from their top females, than what can be achieved by 1-2 MOET flushes per year. In those cases, LOPU-IVEP offers the option of oocyte collection every 2 weeks almost unlimitedly (30 times in the same animals in 4 years).

4. 3. 3. Non-Competitive Applications

This refers to the categories where MOET doesn't work so it is not a reproductive technology that can be applied for exponential multiplication of valuable females. Some of these categories are age-related, specifically the use of LOPU-IVEP for early reproduction of elite females before reaching puberty a sexual maturity, as well as older animals that have become unfertile with age or incapable of carrying a pregnancy themselves but are genetically superior. Other noncompetitive categories refer to animal "conditions", most of them temporary conditions that prevent the animals from producing embryos by MOET for a period, such as early pregnant animals and early postpartum animals. Finally, another category of non-competitive application refers to the practice of LOPU-IVEP in MOET failures, specifically animals with history of repeated failure. These are animals that have been subjected to MOET programs repeatedly and they have always failed to produce transferable embryos for the reason of: (a) repeated luteal regression, (b) failure to super ovulate (non-responders), (c) failure to fertilize (only unfertile eggs are recovered).

Finally, LOPU-IVEP offers opportunities for reproductive rescue of females that have been "crippled" by previous surgeries ending in a uterus with so many adhesions that they can't be flushed anymore, maybe even not getting pregnant anymore. As long as their ovaries are clean, oocytes can be collected and embryos produced *in vitro* to continue propagating their outstanding genetics.

4. 4. Oocyte Retrieval from different category (Donors) of animals

Donor cattle can be in a wide variety of reproductive stages, varying from the prepubertal through the cyclical to the pregnant and the early post-partum animal. Decreasing collection time, minimizing the cost of operation and increasing the operator's control are regarded as important considerations in the development of commercial OPU technology. Oocytes can be harvested from donor cows at any time of the estrous cycle, including the early growth phase of the first follicular wave. The transvaginal ultrasound-guided aspiration procedure can be used to remove dominant follicles (luteal phase) prior to gonadotropin stimulation to enhance the super-stimulation response in donor cows.

4. 4. 1. Oocytes from post-partum cattle

Some workers have examined the possibility of obtaining oocytes from cattle in the early weeks after calving. The use of FSH for oocyte production in early postpartum beef cows that were nursing calves; oocyte collections were performed on day 25 and again on day 35 after calving, on each occasion 18 h after the final FSH injection. They found that FSH cattle yielded a greater number of oocytes.

4. 4. 2. Oocytes from Pregnant Donor Females

Various authors have reported that pregnant cattle have been evaluated as donors of oocytes; this is a novel means of dealing with pregnant female animals of superior genetic quality. One problem with the larger farm animal donor females (*e.g.*, cow, mare) is that their gestation intervals are lengthy in comparison with those of smaller domestic mammals, and that these donor females are out of *in vivo* embryo production during their gestation. However, both cows and mares have been reported to continue follicular wave development during early to mid-gestation. With this in mind, it was deemed logical to take advantage of having these developing follicles available for oocyte harvest during gestation and thus, an attempt was made to collect oocytes from bovine and equine females using transvaginal ultrasound guided aspiration (TUGA) during early pregnancy.

Because the cervix and uterus are not penetrated during the process of aspiration, oocytes can still be collected without disturbing the fetus (**Figure 7**). Donors can be safely aspirated from about 45-100 days of gestation. On rare occasions donors can be aspirated up to 6 months of gestation, as long as the ovaries are accessible to the technician. This application is a good alternative for operations wanting to get a jump on the next generation

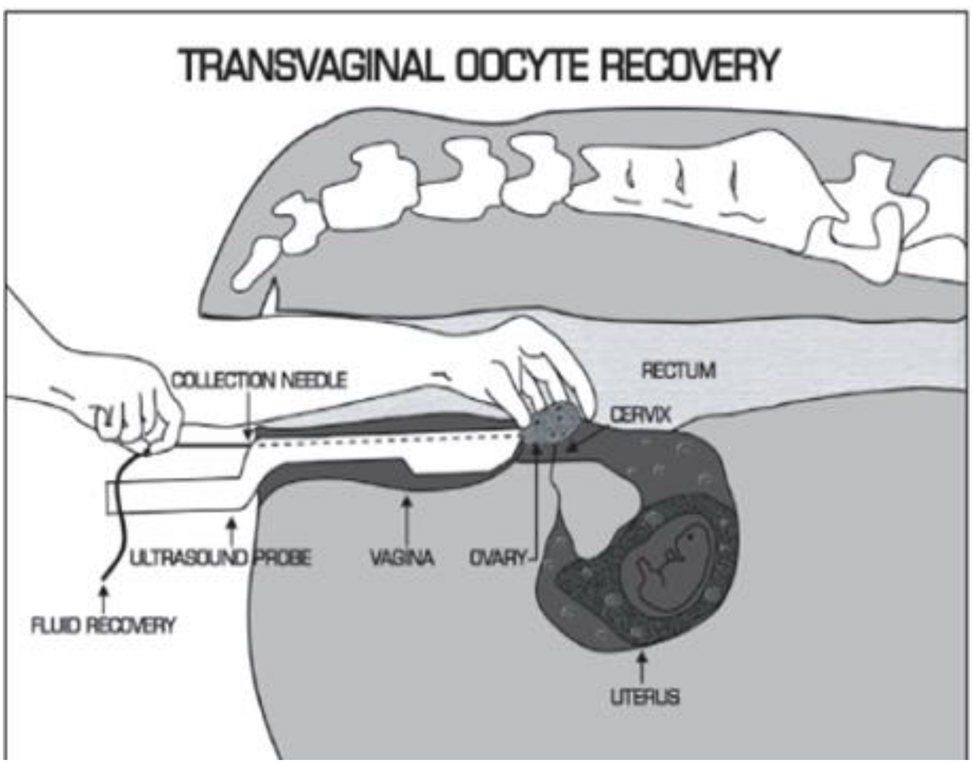

Figure 7. Transvaginal oocyte collection from pregnant cow

4. 4. 3. Oocytes from calves and prepubertal cattle

The interval between generations is one of the key elements in any approach to genetic improvement in cattle; the use of oocytes from young calves offers one way of reducing the generation interval, resulting in faster genetic change. It is known that follicular development occurs in a wave-like pattern in calves as early as 2 weeks of age and that the ovaries contain antral follicles capable of yielding oocytes suitable for maturation and processing into embryos.

It is also known that calf ovaries exhibit larger pools of antral follicles visible on their surface than adult ovaries; this suggests that there is ample potential for recovering numerous oocytes.

In attempting OPU by the transvaginal ultrasound-guided approach, obvious limitations include lack of suitable equipment and the inability of the operator to manually immobilize the ovaries by manipulation *per rectum*. The modified a 5 MHz, convex-array ultrasound transducer designed for intravaginal use in female calves, but were unable to immobilize the ovaries; they were able to collect oocytes from calves in the standing position (10–16 weeks old) or dorsal recumbency (6 weeks old, **Figure 8**).

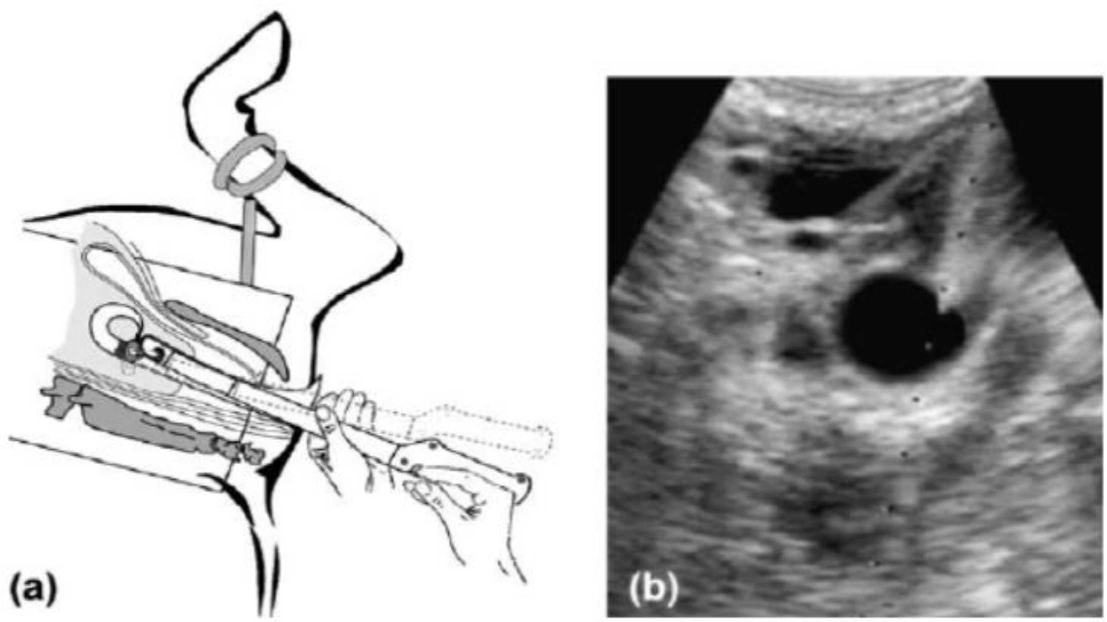

Figure 8. Ultrasound-guided follicle aspiration from calves.

A study by has demonstrated the possibility of recovering oocytes by repeated OPU from 7–12 month-old calves without this affecting growth or puberty. Also examined the possibility of obtaining oocytes from FSH treated calves at 8 and 10 weeks of age and recorded an average of about 26 oocytes collected at 8 weeks and 29 at 10 weeks, resulting in an average of 5.7 pregnancies per calf after embryo production.

5. FACTORS AFFECTING OOCYTE RECOVERY RATE, QUALITY AND DEVELOPMENTAL COMPETENCY

The use of IVP by commercial embryo companies has increased, and currently bovine IVP embryos represent a considerable percentage of the total number of cattle embryos produced in the whole world. However, various factors can affect the success of the technology.

5. 1. Breed

Breed of cattle is one of factors that influences the efficiency of OPU-IVP. It is widely known that *Bos indicus* breeds have a great ability to control their body temperature. This feature may be a genetic adaptation at the cellular level that allows *Bos indicus* cattle to better survive in a hotter climate, resulting in a higher oocyte developmental competence than *Bos taurus* cattle when kept in this environment. The Holstein cows (*Bos taurus*) produced oocytes of lower quality than Brahman cows (*Bos indicus*) during the summer. *B. indicus* breeds have different reproductive attributes in comparison to *Bos taurus* breeds.

5. 2. Follicular size

Different studies have established a relationship between follicle size and oocyte competence; the competence increases as the follicle enlarges, greater rates of embryonic development using oocytes aspirated from follicles greater than 2-3 mm in diameter. The oocyte competence increased in follicles greater than 8 mm. The low developmental rates of oocytes from small follicles may be because they still do not reach complete meiotic and/or cytoplasmic competence, or because they are from follicles already undergoing atresia. Studies made by have shown that oocytes with a diameter of less than 110 μm may still be in the growth phase and are less able to develop after fertilization.

5. 3. Season

Heat stress has been shown to be harmful to bovine oocytes and embryos. Holstein cows have lower reproductive performance in autumn than in winter, which is likely a late effect of high temperatures during the summer. Similarly, oocytes obtained at the beginning of autumn are of low quality, and quality improves gradually as the winter approaches.

5. 4. Body condition and nutritional considerations

The oocyte quality in cattle after they were condition-scored (scale 1–9); the ovaries of thin cows (scores 3–4) had significantly fewer small follicles than cattle in good condition (scores 5–6). From this they recommended that cows in good condition should be selected for Oocyte retrieval. Also, recorded evidence suggesting greater competence in oocytes from dairy cows with a high body-condition score than those with a low score.

The developmental competence of oocytes from small follicles (< 4 mm) of non-superovulated heifers was compromised by excess dietary energy whereas oocytes from medium-sized antral follicles (> 4–8 mm diameter) appeared to be particularly sensitive to rumen-degradable nitrogen. It appeared that oocyte competence could be adversely affected by nutritional regimens that resulted in high circulating levels of ammonia in the plasma.

5. 5. Age of animal

Although antral follicles containing fully grown oocytes are present in the ovaries of the calf at birth, attempts to use these oocytes in embryo production have usually proved to be disappointing. Oocyte developmental competence, which seems to be dependent on age of the donor, is lower in prepubertal heifers than in cows. Prepubertal Holstein heifers between 7-11 months of age have produced oocytes with similar competence to those of cows while oocytes from 3-4- month-old calves were less competent than oocytes from their adult counterparts.

6. CONCLUSION AND RECOMMENDATIONS

This review shows that several are being currently applied commercially in the cattle industry with acceptable results. Oocytes for IVPE can be recovered from the ovaries of slaughtered donors or from live. Various techniques have been used to collect oocytes aseptically from the bovine ovaries. Oocyte slicing, aspiration, dissection and aspiration plus slicing are the most common oocyte retrieval techniques from slaughterhouse ovary. On the other hand, intravaginal oocyte aspiration (OPU) in large animal and laparoscopic method of oocyte aspiration (LS-OPU) in small animal (including shot and wild animals) most commonly used technique in live animals.

Oocyte donor cattle can be in a wide variety of reproductive stages, varying from the prepubertal through the cyclical to the pregnant and the early post-partum animal. Oocytes can be harvested from donor cows at any time of the estrous cycle, including the early growth phase of the first follicular wave.

Virtually all female cattle starting from 2 months of age can be oocyte donors by follicular aspiration. The only exceptions are pregnant animals after the third or fourth month of pregnancy and animals with severe ovarian hypoplasia or in the immediate post-partum before ovarian activity are restored.

Therefore, based on the above conclusion the following recommendations are forwarded:

- IVEP should be adopted both in governmental and private sector of livestock industry; hence, it is an extremely versatile technique because it can be applied to donors of all ages from two-month-old calves to very old cows.
- Since technique of oocyte retrieval from slaughterhouse has their own limitation in terms of contamination, quality and recovery rate of oocyte, the technician should select and use the best technique accordingly.
- Since OPU can have its own therapeutic effect on infertile donors, especially those affected by ovarian cysts, it is the only option and recommended technologies to be applied.
- In live animal, Follicle aspiration is an invasive procedure that can cause bleeding and infection which can create ovarian adhesion and requires a highly skilled veterinarian to perform.
- Hence IVEP of ART was developed in animal science and is a flexible technique, technician should have realm of reproductive physiology and vet school should be committed in teaching the procedure especially in developing countries like Ethiopia.
- Obviously, oocyte collection from live donor by OPU is influenced by various factors; technician should consider each factor to maximize the oocyte recovery rate.

References

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

[2] Alexander B., *et al*. Recent advances in reproductive biotechnology in sheep and goats. *Journal of Veterinary Science and Technology* 1 (2010) 101

[3] Al-Katanani, Y.M., Paula-Lopes, F.F. and Hansen, P.J. Effect of season and exposure to heat stress on oocyte competence in Holstein cows. *Journal of Dairy Science* 85 (2002a) 390-396

[4] Arlotto, T., Schwartz, J.L., First, N.L. and Leibfried-Rutledge, M.L. Aspects of follicle and oocyte stage that affect *in vitro* maturation and development of bovine oocytes. *Theriogenology* 45 (1996) 943-956

[5] Armstrong, D.G., McEvoy, T.G., Baxter, G., Robinson, J.J., Hogg, C.O., *et al*. Effect of Dietary Energy and Protein on Bovine Follicular Dynamics and Embryo Production *In vitro*: Associations with the Ovarian Insulin-Like Growth Factor System. *Biol Reprod* 64 (2001) 1624-1632

[6] Besenfelder, U., Havlicek, V., Mosslacher, G. and Brem, G. Collection of tubal stage bovine embryos by means of endoscopy: a technique report. *Theriogenology* 55 (2001): 837- 845

[7] Bilodeau-Goeseels S, Panich P. Effects of oocyte quality on development and transcriptional activity in early bovine embryos. *Anim Reprod Sci.* 2002 Jun 17; 71(3-4): 143-55. doi: 10.1016/s0378-4320(01)00188-9. PMID: 12047924

[8] Brogliatti, G., Cerrate, H., Caballero, J., Cattaneo, L., Cantarelli, A., Feula, P., Dalla Lasta, M., Ferre, L., Panarace, M. and Medina, M. Pregnancy rates and first-born calves by artificial insemination using sexed semen in Argentina. *Theriogenology* 57 (2002) 369

[9] Brogliatti, G.M. and Adams, G.P. (1996) Ultrasound-guided transvaginal oocyte collection in prepubertal calves. *Theriogenology* 45 (1966): 1163-1176

[10] Carolan, C., Monaghan, P., Gallgher, M. and Gordon, I. Effect of recovery method on yield of bovine oocytes per ovary and their developmental competence after maturation, fertilization and culture in vitro. *Theriogenology*, 41 (1994): 1061-1068.

[11] Chastant-Maillard, S., Quinton, H., Lauffenburger, J., Cordonnier-Lefort, N., Richard, C., *et al*. Consequences of transvaginal follicular puncture on well-being in cows. *Reproduction* 125 (2003) 555-563

[12] Fair, T., Hyttel, P., and Greve, T. Bovine oocyte diameter in relation to maturational competence and transcriptional activity. *Mol. Reprod. Dev*. 42 (1995): 437-442.

[13] Farin, P.W., Piedrahita, J.A., Farin, C.E. Errors in development of fetuses and placentas from in vitro-produced bovine embryos. *Theriogenology* 65 (2006) 178-191

[14] Galli, C., Crotti, G., Notari, C., Turini, P., Duchi, R. and Lazzari, G. Embryo production by ovum pick up from live donors. *Theriogenology* 55 (2001) 1341-1357

[15] Garg, S., Dutta, R., Malakar, D., Jena, M.K., Kumar, D., Sahu, S., and Prakash, B. Cardiomyocytes rhythmically beating generated from goat embryonic stem cell. *Theriogenology* 77 (2012) 829-839

[16] P.C. Goswami , S.Z. Ali , M.A.M.Y. Khandoker , S.A. Azmal , M.K. Alam and R. Khatun , 2004. Collection and Grading of Bovine Cumulus-oocyte-complexes (COCs) from Slaughter House Ovaries in View of *in vitro* Maturation, Fertilization and Culture.

Pakistan Journal of Biological Sciences, 7: 1777-1781. DOI: 10.3923/pjbs.2004.1777.1781

[17] Hammad, M.E., Gabr, S.A., El-Ratel, E.T., Gad, M.A. *et al*. Efficacy of different collection techniques on yield and quality of Egyptian buffalo oocytes. *J. Animal and Poultry Prod*. 5(7) (2014) 413-422

[18] Hasler, J.F., Henderson, W.B., Hurtgen, P.J., Jin, Z.Q., McCauley, A.D., Mower, S.A., Neely, B., Shuey, L.S., Stokes, J.E., Trimmer, S.A. Production, freezing and transfer of bovine IVF embryos and subsequent calving results. *Theriogenology* 43 (1995) 141-152

[19] Hasler, J.F. The current status and future of commercial embryo transfer in cattle. *Anim. Reprod. Sci*. 79 (2004) 245-64

[20] Hendriksen, P.J., Vos, P.L., Steenweg, W.N., Bevers, M.M., and Dieleman, S.J. Bovine follicular development and its effect on the *in vitro* competence of oocytes. *Theriogenology* 53 (2000) 11-20

[21] Hyttel, P., Fair, T., Callesen, H., Greve, T. Oocyte growth, capacitation and final maturation in cattle. *Theriogenology*, 47 (1977) 23-32

[22] Izquierdo, D., Villamediana, P., Lopez Bejar, M. and Paramio, M.T. Effect of *in vitro* and *in vivo* culture on embryo development from prepubertal goat IVM–IVF oocytes. *Theriogenology*, 57 (2002) 1431-1441

[23] Kakkassery, M.P., Vijayakumaran, V., Sreekumaran, T. Effect of cumulus oocyte complex morphology on invitro maturation of bovine oocyte. *J. Vet. Anim. Sci*. 41 (2010) 12-17

[24] Kelly, J.M., Boerema, M. and Walker, S.K. Repeat oocyte collection increases embryo production from juvenile calves. In: Proceeding 14 th International Congress on Animal Reproduction, Stockholm, Sweden, Vol. 2, 2000, p. 110

[25] Kruip, Th. A. M., Boni, R., Wurth, Y.A., Roelofsen, M.W.M., Pieterse, M.C. Potential use ovum pick-up for embryo production and breeding in cattle. *Theriogenology* 42 (1994) 675-684

[26] Kruip, Th. A. M., Bevers, M.M., Kemp, B. Environment of oocyte and embryo determines health of IVP offspring. *Theriogenology* 53 (2000) 611-618

[27] Lazzari, G. & Galli, C. *In vitro* embryos production from valuable cows slaughtered for reproductive failure or terminal illness. Report of the 9th Scientific Meeting of the European Embryo Transfer Association, 1993, pp. 87-99

[28] Lazzari, G. & Galli, C. In vitro embryo production and its application to cattle breeding. Report of the 12th Scientific Meeting of the European Embryo Transfer Association, 1966, pp, 73-82

[29] Majerus, V., De Roover, R., Etienne, D., Kaidi, S., Massip, A., Dessy, F., Donnay, I. Embryo production by ovum pick up in unstimulated calves before and after puberty. *Theriogenology* 52 (1999) 1169-1179

[30] Mamy, K., Atiqah, N.F., Ariani, N. Effect of ovarian types and collection technique on the number of follicles and the quality of cumulus-oocyte complexes in cow. *Bangladesh Journal of Animal Science* 45(3) (2017) 10-16

[31] McEvoy, T.G. Manipulation of domestic animal embryos and implications for development. *Reprod. Dom. Anim*. 38 (2003) 268-275

[32] Palma, G.A., Tortonese, D.J., Sinowatz, F. Developmental capacity *in vitro* of prepubertal oocytes. *Anat. Histol. Embryol*. 30 (2001) 295-300

[33] Raghu, H.M., Nandi, S., Reddy, S.M. Follicle size and oocyte diameter in relation to developmental competence of buffalo oocytes *in vitro. Reproduction, Fertility and Development*. 14(1-2) (2002) 55-61

[34] Rao, M. and Mahesh, U.Y. Efficacy of different harvesting techniques on oocyte retrieval from buffalo ovaries. *Buffalo Bulletin* 31 (4) (2012) 209-213

[35] Roth, Z., Arav, A., B R., *et al*. "Cryopreservation of Sheep Produced Embryos – Current and Future Perspectives" or, A., Zeron, Y., Braw-Tal, R. and Wolfenson, D. (2001) Improvement of quality of oocytes collected in the autumn by enhanced removal of impaired follicles from previously heat-stressed cows. *Reproduction* 122 (2001) 737-744

[36] Saleh, W.M. Assessment of different methods of bovine oocytes collection, maturation and in vitro fertilization of abattoir specimens. *Iraqi Journal of Veterinary Sciences* 31(1) (2017) 55-65

[37] Sayid Ali, Alemayehu Lemma, Tamrat Degefa, Jeilu Jemal, Effect of breed, parity and frequency of collection on quality and quantity of OPU derived oocytes. *World News of Natural Sciences* 34 (2021) 88-97

[38] Seré, C., van de Zijpp, A., Persley, G. and Rege, E. Animal Genetic Resources. *Information Bulletin* 42 (2008) 3-27

[39] Sonowal, J., Barua, P.M., Borah, P., Borgohain, I., Gogoi, C., Deuri, N., *et al*. Effect of L-Ascorbic acid on *in-vitro* maturation of vitrified-Thawed bovine oocytes. *International Journal of Chemical Studies* 5(5) (2017b) 1646-1648

[40] Sonowal, J., Barua, P.M., Borah, P., Dutta, D.J., Hazarika, G., Gogoi, C., *et al*. Effect of α-tocopherol and l-ascorbic acid on *in-vitro* maturation of vitrified bovine oocytes. *International Journal of Chemical Studies* 5(5) (2017a) 1359-1362

[41] Suresh, K., Maurya, S.N. Evaluation of oocyte retrieval methods from bubaline ovaries. *Indian J. Anim. Res*. 34(2) (2000) 130-132

[42] Suthar, V.S. and Shah, R.G. Bovine *In vitro* Embryo Production: An Overview. *Veterinary World* 2(12) (2009) 478-479.

[43] Tervit, H.R. Laparoscopy/laparotomy oocyte recovery and juvenile breeding. *Animal Reproduction Science* 42(1-4) (1996) 227-238.

[44] Thibier, M. Data Retrieval Committee Annual Report, Year 2004. *JETS News* 23 (2005) 11-17

[45] Dawson, J. Transvaginal OPU and IVFEP, Part 1 - future of bovine genetics? Advantage of OPU/IVFEP, *Vet Times* 198 (2017) 1-8

[46] Toni, D., *et al*. Laparoscopy promising tool for improvement of reproductive efficiency of small ruminants. *Macedonian Veterinary Researches* 35(1) (2013) 5-11

[47] Vajta, G., Holm, P., Greve, T. and Callesen, H. Overall efficiency of *in vitro* embryo production and vitrification in cattle. *Theriogenology* 45 (1996): 683-689

[48] Viana, J.H.M., Camargo, L.S.A. A historical perspective of embryo-related technologies in South America. *Anim. Reprod*. 15 (2019) 963-970. DOI: 10.21451/1984-3143-AR2018-0016

[49] Viana, J.H.M., Camargo, L.S.A., 2007. Bovine embryo production in Brazil: a new scenario. *Acta Sci. Vet*. 35 (2007) 915-924

[50] Wolfenson, D., Roth, Z. and Meidan, R. (2000) Impaired reproduction in heat-stressed cattle: basic and applied aspects. *Animal Reproduction Science* 60/61 (2000) 535-547

[51] Zarcula, S.M., Cernescu, H., Godja, G. and Igna, V. Effects of recovering bovine oocyte methods on quantity and quality of cumulus-oocyte complexes. *Adv. Res. Scientific Area-Virtual Conf.* 1 (2012) 2171-2174