The Effect of Different Ovarian Storage Times and Oocyte Collection Methods on the Quantity, Quality, and Maturation of Buffalo Oocytes *In Vitro*

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ABSTRAK

Hendri, Udin Z, Masrizal, Jaswandi, Nanda S. 2024. Pengaruh berbagai waktu penyimpanan dan metode koleksi oosit terhadap kuantital, kualitas dan maturasi *in vitro* kerbau. JITV 29(2):67-78. DOI: http://dx.doi.org/10.14334/jitv.v29i2.3192.

Penelitian ini bertujuan untuk mengetahui pengaruh metode koleksi oosit dan lama penyimpanan terhadap jumlah, kualitas, dan kecepatan maturasi oosit kerbau secara *in vitro*. Ovarium dikoleksi dari rumah potong hewan pada empat waktu penyimpanan yang berbeda (0, 3, 6, 9 dan 12 jam) sebelum diproses dengan tiga teknik: slicing, aspirasi, serta gabungan slicing dan aspirasi. Menurut morfologi lapisan sel kumulus yang melekat erat pada zona pelusida oosit dan penampakan sitoplasma oosit, oosit dibagi menjadi 4 kelompok: A, B, C, dan D. Kompleks kumulus-oosit (COCs) dipindahkan ke media maturasi *in vitro* (IVM) TCM-199 (Sigma) selama 24 jam. Tahapan pematangan inti diperiksa dengan pewarnaan COC yang diperbesar dari masing-masing kelompok. Hasil penelitian menunjukkan bahwa rata-rata jumlah oosit per ovarium berkisar antara 8,08±1,28 hingga 9,83 ±1,89 dan tidak berpengaruh nyata (P>0,05) terhadap jumlah oosit per ovarium. Rata-rata kualitas oosit per ovarium yang berkisar antara 0,2 hingga 5,00 merupakan pengaruh yang sangat nyata antara lama penyimpanan terhadap kualitas oosit (P<0,01). Tingkat pematangan pada stadium M-II berkisar antara 1,33-3,92 per ovarium, dan lama penyimpanan berpengaruh nyata (P<0,05) terhadap tingkat pematangan kerbau lokal. Cara pengambilan yang dilakukan mempunyai pengaruh yang sangat nyata (P<0,01) terhadap jumlah oosit antara pemotongan dan aspirasi, juga antara aspirasi plus pemotongan, dan tidak berbeda nyata (P>0,05) antara pemotongan dan aspirasi plus pemotongan. Rata-rata kualitas oosit berkisar antara 0,10-4,95 per ovarium dan tertinggi pada metode slicing plus aspirasi (P>0,05). Rata-rata tingkat pematangan pada stadium M-II berkisar antara 1,00 hingga 3,85 oosit per ovarium, dan metode pengumpulan oosit berpengaruh signifikan (P<0,05) terhadap tingkat pematangan kerbau lokal. Disimpulkan bahwa waktu penyimpanan hingga 6 jam dan aspirasi plus pengirisan cocok untuk pematangan in vitro kerbau lokal.

Kata Kunci: Kerbau, Oosit, Waktu Penyimpanan Oavrium, Teknis Koleksi, Maturasi *In Vitro*

ABSTRACT

Hendri, Udin Z, Masrizal, Jaswandi, Nanda S. 2024. The effect of different ovarian storage times and oocyte collection methods on the quantity, quality, and maturation of buffalo oocytes *in vitro*. JITV 29(2):67-78. DOI: http://dx.doi.org/10.14334/jitv.v29i2.3192.

This study aimed to ascertain the impact of oocyte harvest methods and storage times on the amount, quality, and rate of buffalo oocyte maturation in vitro. Ovaries were collected from slaughterhouse at four different time storages (0, 3, 6, 9 and 12 h) before processing with three collection techniques slicing, aspiration, and slicing plus the aspiration of oocytes. According to the morphology of cumulus cell layers closely attached to the zona pellucida of the oocytes and the cytoplasmic appearance of the oocytes, the oocytes were divided into 4 groups (A, B, C, and D). Cumulus-oocyte-complexes (COCs) were transferred into in vitro maturation (IVM) medium of TCM- 199 (Sigma) for 24 h. The stages of nuclear maturation were examined by staining the enlarged COCs from each group. The result found that the average quantity of oocytes per ovary ranged from 8.08±1.28 to 9.83 ±1.89 and no significant effect (P>0.05) on the quantity of oocytes per ovary. The average of oocytes quality per ovary ranges from 0.2 to 5.00 was a highly significant effect of storage time on quality oocytes (P<0.01). The maturation rate in the M-II stage ranges from 1.33 to 3.92 per ovary, and storage time is a significant effect (P<0.05) on the maturation rate of local buffalo. The collection methods were highly significant (P<0.01) on the quantity of oocytes between slicing and aspiration, also between aspiration plus slicing, and no significant (P>0.05) between slicing and aspiration plus slicing. The Average oocyte quality ranged from 0.10 to 4.95 per ovary and the highest in slicing plus aspiration methods (P>0.05). The average maturation rate in M-II stage was ranged from 1.00 to 3.85 oocytes per ovary, and the methods of oocytes collection were significant (P<0.05) on the maturation rate of local buffalo. In conclusion that the storage time of up to 6 h and aspiration plus slicing is suitable on in vitro maturation of local buffalo.

Key Words: Buffalo, Oocyte, Ovary Storage Time, Collection Techniques, *In Vitro* Maturation

INTRODUCTION

The population of buffalo in west Sumatra consists of indigenous swamp buffalo or local buffalo, and almost all these buffalo are reared by smallholder farmers. The Buffalos had poor reproductive performance, silent estrus symptoms, seasonal anestrus, delayed adolescence, delayed first calving, delayed postpartum conception, a long calving gap, and a low total number of follicles in the ovary, among other reproductive issues (Fitriani et al. 2023). The main issue with buffalo is low reproductive efficiency, indicated by low conception rate and high mortality. Countries have prioritized improve the genetic potential of swamp buffalo for meat production. The research on buffalo has been focused on assisted reproductive technologies (ARTs), including in vitro fertilization (IVF) and in vitro maturation (IVM). ATR might be useful for enhancing the state of cattle reproductive at the moment (Srirattana et al. 2022).

Buffalo embryo in vitro development has proved unsuccessful due to a number of issues, including insufficient follicles on the ovaries and a low rate of oocyte recovery. Oocytes are matured in vitro (IVM), sperm are activated, matured oocytes are fertilized in vitro (IVF), and fertilized oocytes are then cultured in vitro (IVC) until the blastocyst stage. Events that take place during oocyte maturation, fertilization, and the subsequent growth of the fertilized oocytes have a significant impact on in vitro embryo development. In vitro maturation (IVM) is used in assisted reproductive technology to prepare oocytes for in vitro fertilization. The first and most crucial step in developing quality and quantity-conscious embryos in vitro is the careful recovery of oocytes. The time between collection and delivery to the laboratory within 4 hours of the slaughterhouse is not always easily managed. Only high-quality oocytes were selected for in vitro maturation because a higher proportion of high-quality oocytes promotes a faster rate of cumulus growth and maturation (Rajesh et al. 2018). It is feasible to increase the tolerance time for ovary collection by up to 6 hours and increase the effectiveness of the IVEP by ensuring that the temperature of the ovarium is kept below 30°C throughout transit (Francesco et al. 2007). In contrast to other preservation periods of up to 2 hours, (Abdel-Khalek et al. 2010)found that preserving ovaries at 25°C for 5–6 hours successfully sustained the oocytes' quality and recovery rate. For the generation of blastocysts on day 7 of IVC, shipping bovine oocytes from slaughterhouses for either 18 or 24 hours was preferable to the standard maturation approach (Barceló-Fimbres et al. 2015).

Domestic animals have been used to extract oocytes utilizing a variety of techniques, including ovary slicing and post-aspiration slicing of ovarian follicles. However, the effectiveness of these techniques varied (Udin et al. 2020; Rajendar et al. 2024). The oocytes from the abattoir ovaries are harvested using a variety of procedures. The aspiration, slicing, and penetration methods are among them (Saleh 2017). That may suggest the effectiveness of the slicing approach as a collecting strategy for the quantity and quality of buffalo oocytes (Gabr et al. 2014). Buffalo oocyte in vitro maturation was also enhanced in TCM-199 with hormone additions. The method of oocyte harvest for IVM is also the first and most important step toward effective in vitro embryo formation (Segers et al. 2015). The slicing approach, in accordance with (Habeeb and Hussain 2018), produced a much higher quantity of buffalo oocytes 220 distribution grade A (90), B (50), and C. (80). The better procedure for recovering an oocyte for additional (*in vitro*) research is aspiration (Singh et al. 2018). The goal of the current study was to establish an appropriate window of time between the slaughterhouse and the laboratory's ovarian recovery method and technique for collecting local buffalo oocytes.

MATERIALS AND METHODS

Chemical and media

Ovaries from Slaughterhouse buffalo in saline solution for transportation ovaries PBS (phosphate buffer solution) for handling, washing, and evaluation oocytes. Medium maturation TCM-199 + BSA (bovine serum albumin) + hormones from Sigma chemical C0, USA. Gentamicin (50 µg/ml) was added to the medium used for transportation, washing of the ovaries, collecting the oocytes, and maturing the oocytes before use. Before use, handling, collection, and maturation media were equilibrated with 5% CO_2 in a humidified atmosphere at 38°C for at least 2 hours. The maturation grade was then determined using the fixation solution of ethanol and acetic acid.

Experiment-1. Effect of ovaries stored for a period of time on the amount, quality, and maturation of oocytes from local buffalo

Total of 60 ovaries from a local buffalo slaughterhouse were used in this investigation for five groups at different times. Ovarian storage times were divided into five groups in the lab, including 0 hours, 3 hours, 6 hours, 9 hours, and 12 hours. In each group, there were 12 ovaries.

Experiment-2: Effect of collecting method on local buffalo oocyte quantity, quality, and maturation rate.

Oocytes were removed from 60 ovaries using three different techniques: aspiration, slicing, and slicing with

aspiration. Twenty ovaries were used in each oocyte collection technique.

Collection of ovaries

As soon as the buffalo was slaughtered, the ovaries were taken from the nearby slaughterhouse in Padang, West Sumatra. The ovaries were transported to the lab in a thermos filled with normal saline (0.9%) containing gentamicin and maintained between 27 – 30°C. Ovaries from slaughterhouses were split into five groups for storage time: 0 hours, 3 hours, 6 hours, 9 hours, and 12 hours. These groups were processed in the lab. There were 60 ovaries overall, divided into 12 groups.

Collection methods of oocytes

Oocytes were removed from the ovaries using three techniques: aspiration and slicing-aspiration. The ovaries were put in a clean glass petri dish with 2 cc of PBS. Aspiration and slicing-aspiration were the methods employed to collect oocytes. Each collection technique consisted of 20 ovaries, and the total was 60 ovaries Aspiration technique: The ovaries aspiration from individual ovaries after carefully washing to remove extraneous tissue and placed in a Petri dish containing 1 ml of PBS. Oocytes were aspiration from the visible follicle on the ovarian surface with a 22gauge needle fixed to a 5 ml of disposable syringe. Slicing procedure: Using forceps, the ovaries were held firmly in a sterile glass Petri dish with 2 cc of PBS. The blade was attached to the artery forceps and used to cut the ovaries into potentially tiny parts. The PBS medium-containing oocyte was put in a Petri dish and inspected using a stereomicroscope. Combining aspiration and slicing: The aspirated ovaries underwent additional slicing to count the remaining oocytes (post-aspiration slicing), and the number of oocytes obtained using each approach was then counted. The oocytes were microscopically examined, after which they were moved to a grading plate with PBS. The PBS medium was made, and 2 ml of BSA was added to each ml of PBS. A 0.22 µm Millipore filter was used to filter the medium after the pH medium was adjusted to 7.2 to 7.4 and the osmolarity to 280 to 300 ml Osmol kg^{-1}.

Evaluation of oocytes

Five hundred twenty-one oocytes were collected using three different procedures, and 522 oocytes were stored in five different groups of storage duration. According to (Ebrahimi et al. 2010), oocytes were examined under stereomicroscope and divided into four categories: (1) cumulus oocytes-complexes (COCs) with compact cumulus cell (3 layers and homogenous ooplasm, (2) expanded cumulus cell oocytes, (3) denuded oocytes with completely devoid cumulus cells and heterogeneity ooplasm, and (4) partial.

***In vitro* maturation**

The oocytes were matured in TCM-199 maturation media supplemented with 0.3% BSA, 100 IU penicillin G, 100 µg streptomycin/ml, and the hormone FSH at a concentration of 0.04 ml. The 0.22 µm Millipore filter was used to filter the medium. 500 µl of the ready-made maturation media were put into a three-well dish for storage, and three different methods of oocyte collection using sterile mineral oil. The medium was incubated in a CO_2 incubator (5% CO_2, at 38°C with saturated humidity) for at least 60 min to achieve temperature and gas equilibrium before the oocytes were placed on culture dishes. Before being cultured in the intended maturation medium, the COCs were washed three times in the washing medium and twice in it. The maturation period was 24 hours and 10-20 oocytes were placed in maturation media, liquid paraffin oil was added on top, and the Petri dishes were then moved into a CO_2 incubator. In experiment-1, there were 247 oocytes (grade A and B), and in experiment-2, there were 247 oocytes (grade A and B) collected using three different methods.

Fixation, staining, and examination of oocytes

Oocytes were washed in PBS containing 1 mg/ml of hyaluronidase to get rid of the cumulus cells after the 24-hour maturation phase. Oocytes were then placed onto a clean slide after being washed twice in PBS treated with 2% BSA. Overnight, slides were soaked in a fixation solution (three ethanol to one glacial acetic acid). Then, using phase-contrast microscopy, oocytes were stained with 1% orcein in 45% acetic acid to detect different stages of maturation, such as the germinal vesicle (GV), which indicates that chromosomes are enclosed within a nuclear membrane, the germinal vesicle break down (GVBD), which shows the absence of a visible nuclear membrane and chromatin condensation. Metaphase-1 (M1): Chromosomes are condensed in pairs without a polar body being visible; Metaphase-2 (M2): Chromosomes have formed an equatorial plate, and the remaining chromosomes are either substantially condensed or have the first polar body or mature oocytes, extruded from them; Degenerated (Deg) is an example of vacuolated oocytes with constricted cytoplasm or chromatin.

Statistical analysis

The obtained data were statistically evaluated using SPSS 16 and the chi-square test to compare two treatments at each experiment stage.

RESULT AND DISCUSSION

Experiment-1: Effect of ovaries stored for a period of time on the amount, quality, and maturation of oocytes from local buffalo

The average number of oocytes per ovary was 9.833±1.89, 8.03±1.28, 8.75±1.72, 8.33±2.46, and 8.41±1.92 in storage periods of 0 hours, 3 hours, 6 hours, 9 hours, and 12 hours, respectively (Fig. 1). The amount of buffalo oocytes was not significantly affected by storage time ($P > 0.05$). At 0 and 12 hours, grade A oocyte quality ranged from 1.7% to 5.9% on average. The highest-quality oocytes were grade B for all oocyte collection methods, and the average oocyte quality was 28.0% at 9 hours, 32.7% at 12 hours, 46.4% at 3 hours, 50.8% at 0 and 6 hours, and 57.1% at 6 hours (Fig. 2). The average grade C oocyte quality ranged from 10.5% at 6 hours to 25.8% at 12 hours. For grade D, the average oocyte quality increased after 9 hours and decreased after 3 hours of ovarium preservation. The quality of the local buffalo's oocytes is significantly affected by storage duration ($P<0.05$). The percentage of oocytes that matured was lower in GV; it ranged from 8.20% at 3 hours of storage time to 28% at 12 hours, whereas it ranged from 0.1% at 0 hours of storage time to 25.60% at 12 hours. The average M-1 storage time ranged from 0.00% at 6 hours to 6.10% at 0 hours. The in vitro maturation percentage for M2 grade was 74.20% at 0 h, 73.50% at 3 h, 72.50% at 6 h, 50.00% at 9 h, and 46.20 at 12 h storage time. In vitro maturation was not affected by storage time between 0 h, 3 h, and 6 h ($P>0.05$) but was affected by storage time between 9 h and 12 h ($P<0.05$). This finding demonstrated that while oocyte number and in vitro maturation % were not significantly impacted by storage time, the quality of native buffalo oocytes was.

Effect of storage time on oocytes quantity of local buffalo

This result found that the quantity of oocytes tends to decrease by prolonging the time storage of ovaries before being processed in the laboratory. This finding, supported by Kandil et al. (2023), indicates that buffalo oocyte developmental ability is not negatively impacted by delaying ovary collection and processing by up to 6 h. The number of oocytes in this study was higher than (Rajesh et al. 2018), who found that the recovery rate for oocytes from ovaries with CL was 1.35%, while that for ovaries without CL was 1.97%, and (Pitroda et al. 2021), who found that the recovery for oocytes from Riverine buffalo ovaries was 2.74 ± 0.194. The oocyte recovery rate was discovered to be 3.12 per ovary by Ruhil and Purohit (2015) , but the presence result was less favorable than that of Abdel-Khalek et al. (2010) found that the average number of oocytes per ovary ranged from 9.9 to 12.4 and that the oocyte recovery rate decreased barely over time, from 68.1% at 5 hours to 78.6% at 9 hours. If the specimen is kept in a cool box between 4 and 8 degrees Celsius after the animal has been slaughtered, it is preferable to transport it there immediately (Saleh 2017). Additionally, it was hypothesized that photoperiod influences buffalo heifer ovarian function independently of dietary and metabolic conditions and that slaughterhouse specimens can be used to acquire more oocytes (Salzano et al. 2019) . In addition, the quantity and quality of oocytes were unaffected by the calorie content of the diet (Sales et al. 2015). The developmental potential of oocytes was considerably ($P<0.05$) decreased after ovaries were kept at 4°C for 12 or 24 hours (Ravindranatha et al. 2003). The geographic location, the amount of processed ovaries, the season of recovery and the recovery technique used, the reproductive condition of the donor

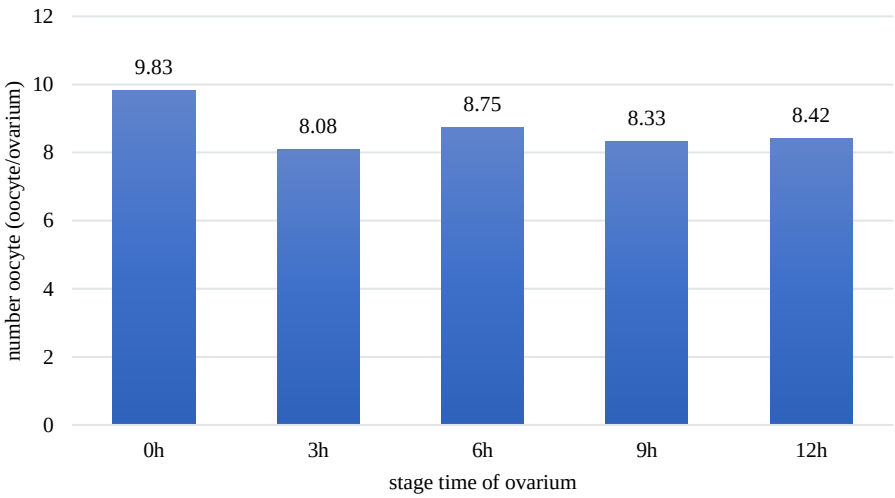

Figure 1. Effect of storage time on quantity of oocytes of local buffalo

animal from which the oocytes were retrieved, and the method used to select ovaries from the slaughterhouse could all contribute to the discrepancies (Al-Shammari and Almeeni 2022). The size of the follicles and other functional features of the ovaries, such as CLs, have been discovered to impact the rate at which oocytes recover in buffalo (Shabankareh et al. 2015) . This study indicated that extending the ovarian storage period to 12 hours was appropriate for the amount of local buffalo oocytes.

Effect of storage time on oocytes quality of local buffalo

The findings that storage time had a significant impact on the quality of buffalo oocytes were confirmed by studies by Saleh (2017), Francesco et al. (2007), and Ravindranatha et al. (2003) that found that storing ovaries at 4°C for 12 or 24 hours significantly (P<0.05) decreased the developmental potential of oocytes. This result's percentage of high-quality oocytes was lower than those in (Saleh 2017): 55%–75% range, Jamil et al. (2008): 50–56% range in autumn and summer, and Abdel-Khalek et al. (2010): range of 42–65.7%. This result demonstrated that prolonged storage times directly impacted oocyte quality. (Saleh 2017) and (Lonergan and Fair 2016) concurred that while several factors directly affect oocyte quality, the time of slaughter has the greatest impact. That element might prevent in vitro oocyte maturation, resulting in low-quality embryos (Aguila et al. 2020). Sonowal et al. (2017) state that good-quality oocytes have more than 4-5 layers of cumulus cells around the zona pellucida and uniform cytoplasmic appearance. When evaluating the in vitro development competence of oocytes, the oocytes quality is crucial (Lemseffer et al. 2022).

This present study was similar to (Sonowal et al. 2017) in that the superior quality grade A +B COCs were 0.2±0.1 to 1.1±0.2, caused the ovarian function in buffalo heifer is fluencies by photoperiod independent of nutritional status and metabolic status. It has been speculated that buffalo abattoir-derived oocytes are particularly affected by cellular damage due to autolytic processes, especially when they reside in excised ovaries for prolonged periods (Kumar et al. 2023). Furthermore, the reproductive status, estrous cycle stage, existence of a CL, and mode of oocyte retrieval all impacted the number of ovarian follicles, yield, and quality of oocytes (Ahmed et al. 2015). Additionally, the frequency for grade I oocytes was significantly higher in the winter and spring (8.31.29 and 9.411.32, respectively) (P<0.05). In the spring (10.52.08), grade II oocyte frequency was greater (P<0.05). In the summer and fall, grades III and IV were slightly higher (P<0.05) (Mansor 2019) .

Effect of storage time of ovary on maturation rate of local buffalo

The maturation rate of local buffalo in this present study was similar with Yousaf and Chohan (2003) was reached the M2 67.1 % and 79.1 % from follicles 4-<6 mm and 6-<8mm, respectively, and Deb et al. 2016) was 74.16 ±5.49 %. The result in the storage time of ovaries up to 6 h was significantly higher in vitro maturation in M2 of local buffalo than Elbaz et al. (2019) was 42.0 % (1.68±0.3) in ovaries with CL and 55.6 % (5.9±1.3) with ovaries without CL, and (Habeeb and Hussain 2018) founded 33.33 % in TCM-199 medium and 13.9% in SOF. (Mohammed et al. 2019) claim that variations in follicular fluid compositions are caused by variances in follicle size, nutrition level, and animal species, which either promote or impede oocyte maturation and subsequent embryonic development. Additionally, the buffalo oocyte maturation rate in the TCM-199 medium was 74.82% (Aquino et al. 2015).

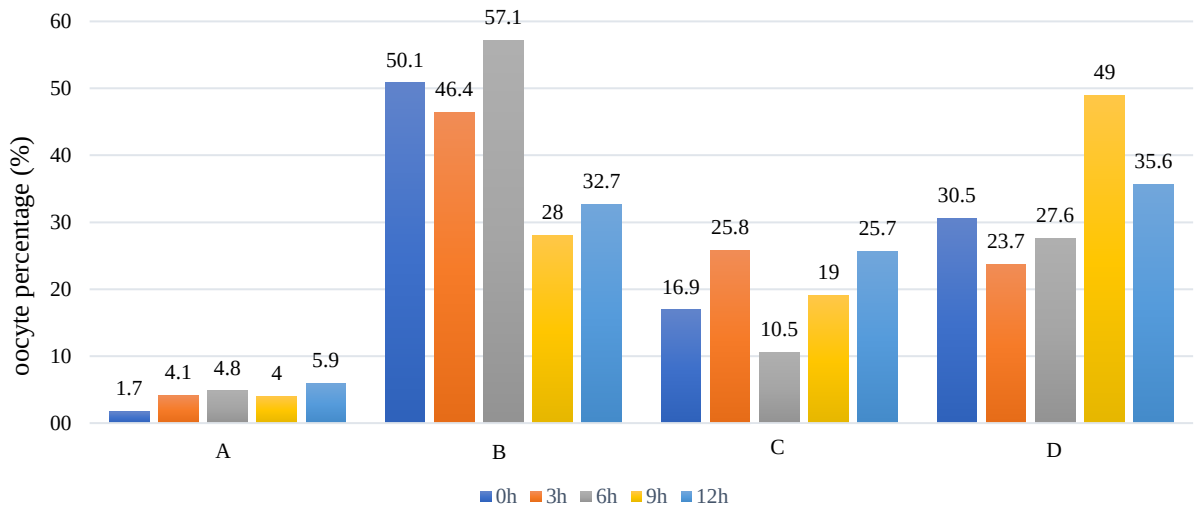

Figure 2. Effect of storage time on percentage of oocytes quality of local buffalo

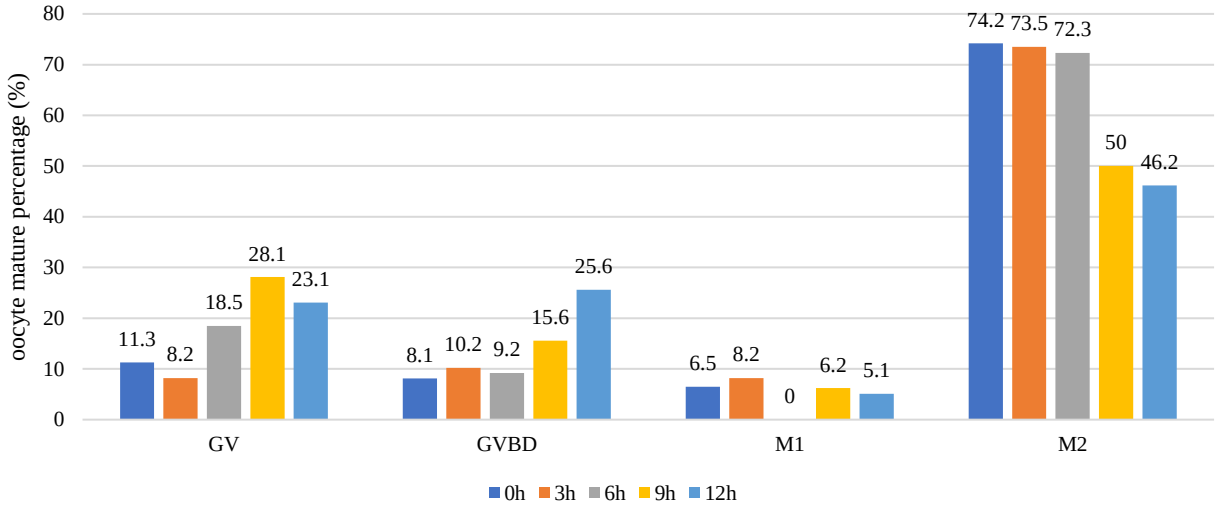

Figure 3. Effect of storage time on *in vitro* maturation percentage of local buffalo

The results of the current study demonstrated that the maturation rate of local buffalo was significantly affected by the length of time between the slaughterhouse and laboratory processing, which was linked to the quality of oocytes recovered from ovaries. In the creation of IVM, the amount and quality of recovered oocytes from each ovary are crucial factors. An oocyte's outward appearance and the amount of cumulus cells it contains have been used to estimate or evaluate its developmental potential, or its capacity to go through typical maturation, fertilization, and development to the blastocyst stage. FBS was superior to EBS for IVM of buffalo oocytes (Puri et al. 2015). EBS may be employed as a serum substitute for oocyte maturation, although the pace was slower than FBS. Therefore, in buffalo, it is possible that ALC influences mitochondrial function, controls paracrine factors produced from oocytes, and boosts steroid hormone production, improving in vitro embryonic development and the quality of developed oocytes (Xu et al. 2018). The results imply that buffalo ovary storage duration is longer in 6 h and there is no tendency to reduce the quantity up to 9 h or 12 h ($P > 0.05$) but the considerable impact on oocyte quality, higher grade at COCs, and maturation rate up to 6 hours of storage.

Experiment-2: Effect of collection technique on quantity, quality, and maturate rate of local buffalo oocytes

The average number of oocytes per ovary using the slicing, aspiration, and slicing plus aspiration procedures was 11.31.63, 4.152.39, and 10.61.2, respectively (Fig.4). Oocyte number per ovary between slicing and aspiration had a highly significant effect ($P<0.01$), whereas oocyte number per ovary between slicing and aspiration plus slicing had no significant effect ($P> 0.05$). Aspiration and aspiration combined with slicing had a substantial ($P<0.05$) impact on the quantity of oocytes in each ovary. According to slicing, aspiration, and aspiration plus slicing, the percentage of oocytes with grade A quality was 3.15%, 2.40%, and 5.70%, respectively. In the three categories of slicing, aspiration, and aspiration plus slicing, the proportion of grade B was 42.5, 37.30, and 46.705, respectively. In the slicing, aspiration, and aspiration plus slicing processes, the percentage of oocytes with grade C quality was 20.4%, 34.10%, and 16.10%, respectively. Slicing, aspiration, and aspiration with slicing each produced 34.10%, 36.10%, and 31.10% of quality oocytes in grade D, respectively (Fig.5). The quality of the buffalo oocytes was not significantly affected ($P>0.05$) by the collection method. In slicing, aspiration, and aspiration plus slicing, respectively, there were 17.50%, 28.20%, and 15.30% of GVs that had undergone in vitro maturation. Sliced, aspirated, and aspirated plus sliced GVBD rates were 10.7%, 18.20%, and 12.60%, respectively. Slicing, aspiration, and aspiration with slicing in M1 were 7.8%, 3.00%, and 2.70%, respectively. According to slicing, aspiration, and aspiration plus slicing, the maturity percentage of M2 was 64.2%, 60.60%, and 69.40%, respectively (Figure.6). The percentage of local buffalo that matured in vitro was not significantly affected by the collection technique ($P>0.05$).

Effect of collection technique on oocyte quantity of local buffalo

This finding supported Gabr et al. (2014) that the slicing technique yielded significantly more oocytes per ovary than follicle by aspiration technique, and Habeeb and Hussain (2018) that the oocyte recovery rate was

better with slicing than the aspiration in buffalo. The slicing methods were higher than aspiration plus slicing and aspiration methods on the quantity of local buffalo. Saleh 2017) asserts that aspiration procedures produce a reasonable number of oocytes with good quality and good embryo production, whereas slicing methods produce more oocytes with intermediate quality. The outcome of the presence research is superior to that of Hammad et al. (2014) found that the number of oocytes per ovary of buffalo was 4.1±0.30, 3.1±0.10, and 3.67±0.27 in slicing, aspiration, and aspiration plus slicing respectively. Puncture was 3.46±0.31, and aspiration methods were 2.38±0.19. Slicing yielded a significant ($P<0.01$) number of oocytes per ovary that was 7.98±0.70. Additionally, it was shown that an ovary without CL was better suited to collecting a greater quantity and higher caliber of COCs (Rajesh et al. 2018). In comparison to the dissection, aspiration, and aspiration plus slicing techniques, the recovery of oocytes utilizing the slicing approach enhanced the oocyte recovery rate. It produced a larger percentage of good-grade oocytes (COCs and enlarged oocytes) (Gabr et al. 2014). By slicing, the mean number of oocytes recovered per ovary was significantly larger (7.88) than it had been using the follicular puncture (3.59) or aspiration (2.50) techniques (P 0.05) (Mahesh et al. 2014). Because some follicles are present that are deeply lodged in the cortex and are freed by slicing the ovary, the study's finding that fewer oocytes were aspirated may be explained by the presence of these follicles. Some oocytes may even be lost when aspirating a follicle, which is not feasible when using the slicing methods. The aspiration approach is the better strategy for recovering oocytes for use in subsequent (in vitro) research, according to a separate (Singh et al. 2018) study.

In the current study, the buffalo ovaries' post-aspiration slicing resulted in an extra 39.15% of oocytes per ovary. This variation in oocyte collection may be caused by the release of cells from the deeper cortex and both surface follicles in slicing techniques (Rose 2014). The fact that certain follicles are deeply entrenched inside the cortexes and are liberated by slicing the ovary may be the cause of the aspiration method's decreased recovery of oocytes. While aspirating follicles, is not possible when employing the slicing procedure, some of the oocytes may even be lost (Gabr et al. 2014). Furthermore, (Saleh 2017) argued that it was ethical to keep the ovarian samples that were aspirated because they may still produce new oocytes by slicing those ovaries. On the other hand, the recovery of buffalo oocytes was unaffected by cutting, aspiration, or a combination of approaches (da-Silva et al. 2021). The reproductive state may be the cause of the various outcomes. This finding established that the method of collecting local buffalo oocytes had a substantial impact on the quantity and that the way of slicing them produced more oocytes from the local buffalo.

Effect collection technique on oocytes quality of local buffalo

The oocytes quality of buffalo in the presence study found that the aspiration plus slicing technique was higher than slicing and aspiration and supported Singh et al. (2018) and (Saleh (2017) that the slicing technique could be considered as the best technique for harvesting the quality of the bovine oocyte. The percentage and quality number per ovary were higher in grade B was 42.5%, 37.30%, to 46.30% in slicing, aspiration, and aspiration plus slicing, respectively (Fig.5). This finding was lower than reported by Gabr et al. (2014) in buffalo was 51.24%, 63.17%, 51.34%, and 42.02% in aspiration plus slicing, slicing, aspiration, and dissection, respectively and Saleh (2017) was 80%, 55%, 45%, and 40% in aspiration, slicing, slicing plus aspiration and slicing, respectively and also the recovery of A+B grade oocyte was 86.01% and 81.76 % by aspiration and slicing technique, respectively (Singh et al. 2018).

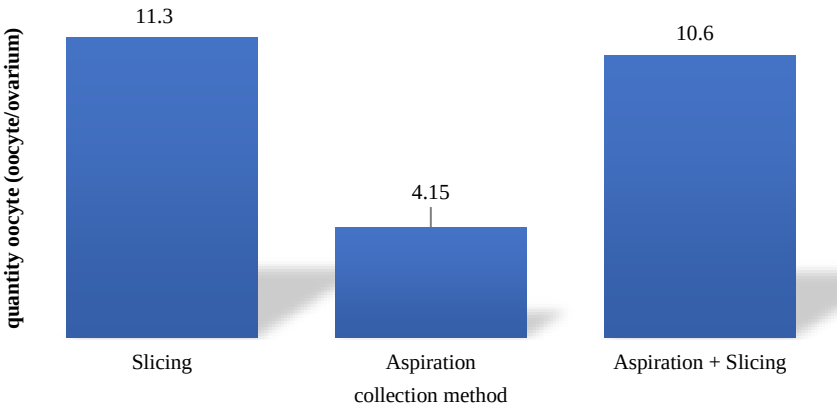

Figure 4. Effect of collection methods on oocytes quantity of local buffalo

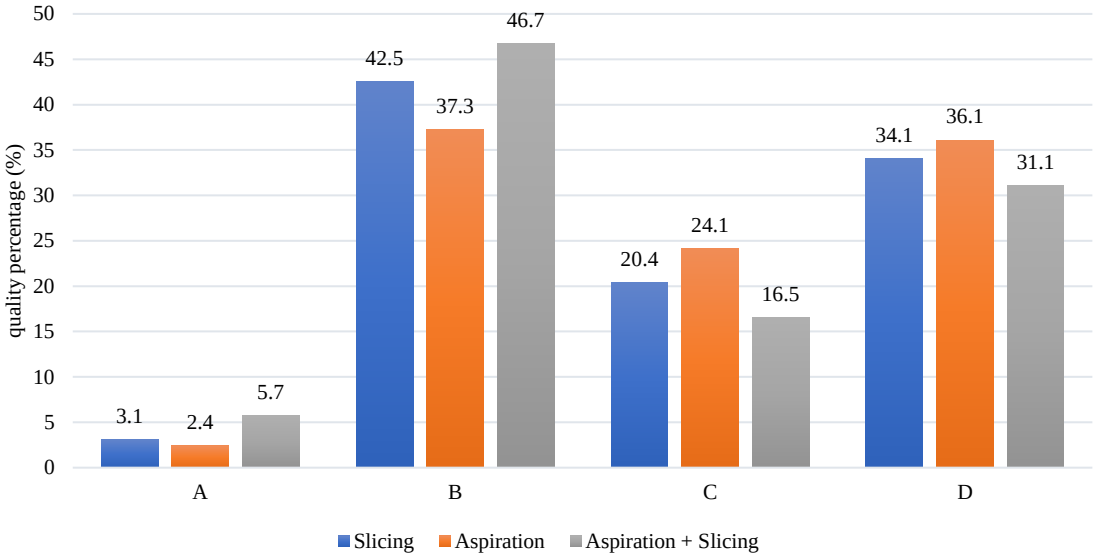

Figure 5. Effect of collection technique on oocytes quality of local buffalo

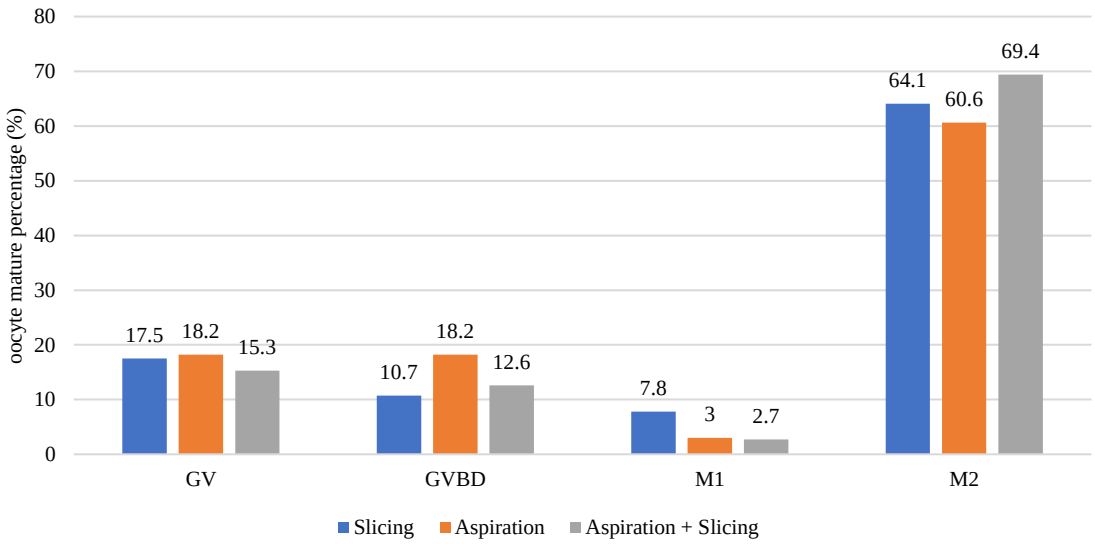

Figure 6. Effect of collection methods of oocytes on maturation percentage of local buffalo

According to Hammad et al. (2014) and (Mahesh et al. 2014), the COCs category was 51.34% in aspiration, 63.17% in slicing, and 51.24% in aspiration plus slicing. The average culture grade A and B COCs recovery was 65.79%. This result was higher than that reported by (Mehmood et al. 2011), who found that the COCs recovery per ovary was 0.9 and 2.2 in aspiration and slicing, respectively, and 0.4 -3,85 good COCs/ovary in buffalo (Nandi et al. 2002), as well as by Jamil et al. (2008), who found that of the three oocyte recovery methods used, dissection produced the highest percentage (36.7). The efficiency of the slicing approach as a collecting method on the amount and quality of buffalo oocytes was also demonstrated byHammad et al. (2014).

The percentage of oocyte quality in the three collection techniques was A grade lower than B, C, and D grade. Aspiration plus slicing technique was higher in A and B grades and least in C and D grades. This finding was supported by Singh et al. (2018), that was no significant difference in the overall recovery rate between the technique, and Ahmed et al. (2015) reported lower recovery rates grade A (38.77%) and B (27.02%). According to Elbaz et al. (2019) that oocytes aspirated from medium-sized follicles (3-6mm) were of higher quality and maturation rate than large (>6 mm) and small-size follicles (≤3 mm). For grade I oocytes. Meanwhile, the slicing method yielded a significantly higher number of oocytes in grades A, B, and C was 40.90 %, 22.7 %, and 36.4%, respectively. While the

aspiration method in grades A, B, and C produced 32%, 28%, and 40% of COCs per ovary, respectively, the slicing approach produced more COCs per ovary (Habeeb and Hussain 2018). The result is different with Mahesh et al. 2014) that the percentage of COCs collected by slicing was higher (P<0.05) at 63.17%, was higher than (P<0.05) than aspiration at 51.34%, aspiration plus slicing was 51.24 %.

Effect of collection technique of oocytes on maturation rate of local buffalo

The maturation rate of COCs at the M2 stage per (A+B) is comparable to that reported by Mehmood et al. (2011), which was 65% in the aspiration technique and 40.0% in slicing technique, and to that reported by Gabr et al. 2014), which was 67% in TCM-199 medium and 58.6 in HamsF10 (P0.05), but higher than that reported by (Saleh 2017), which was 45% in Mendes et al. (2018), findings that the average nuclear maturation in cattle was 58.2% confirm this finding. The result obtained via extrusion of the first polar body is less than that of (Deb et al. 2016), which was 74.16%. Despite a marginally higher percentage of M2 oocytes being found in A-grade COCs obtained by slicing and puncture (84.13.1% and 82.94.7%, respectively) than those obtained by aspiration I and II (78.61.9 and 81.72.5%, respectively), there was no statistically significant difference in the proportion of M2 oocytes after IVM of A grade and B COCs harvested by 4 methods (Vuong et al. 2020). The percentage of M2 oocytes in A grade COCs was not significantly different between the slicing and puncture methods, as well as between the aspiration I and aspiration II methods. The proportion of first polar body extrusion matured in vitro at 56.5°C was 79.5% and 63% (A and B), and at 38,5°C was 85.41% and 74.1%, according to Sen & Kuran (2018) (A and B). In FBS and EBS, the percentage maturation rate was 83.80% and 77.45%, respectively (Puri et al. 2015). Season, oocyte quality, cumulus expansion, maturation, and developmental competence of buffalo oocytes' in vitro maturation all had a significant relationship with one another (Hasbi et al. 2022). According to Gad et al. (2018) , polar body rates (95.8 and 45.5%, respectively) and expansion rates (P<0.05) demonstrated higher maturation rates. Furthermore, the buffalo oocytes' recovery rate, quality, and in vitro maturation all improved with the wintertime, medium-sized follicles, and without CL (Elbaz et al. 2019). These various oocyte collection techniques did not impact the pace of oocyte nuclear maturation (Nikiforov et al. 2020). According to Mohammed et al. (2019), follicle size, nutrition level, and animal species all affect the composition of the follicular fluid, which can either encourage or impede oocyte maturation and continued embryonic development.

According to this study, there is no discernible difference in local buffalo's maturation percentage and rate depending on the method used to harvest the oocytes. According to this finding, which was corroborated by (Mehmood et al. 2011), buffalo oocytes retrieved from resected ovaries using the suction approach had a higher IVM rate than those obtained using the slicing procedures (Chaudhari et al. 2014). Buffalo oocyte maturation is influenced by a wide range of variables, including culture medium, recovery technique, oocyte quality, season of collection, and ovarian condition. It was possible to extract oocytes of every grade using the aspiration approach from samples taken from slaughterhouses, according to Hammood and Aliawy (2017), and it was also possible to mature oocytes in a lab. In contrast to a previous study by Saleh (2017), the suction approach produced fewer oocytes in this study, but they were more mature than those from other procedures. According to (Mahesh et al. 2014), slicing and slicing after aspiration were the earlier methods that produced more oocytes count with good quality that reached maturity condition in a well-considered degree when comparing various methods for oocytes collection in regards to the oocytes number, quality, and degree of maturation. As an alternative to aspiration using a syringe or vacuum pump, Ondho et al. (2024) assert that methods of slicing and puncturing the ovaries can be used. According to follicle size, nutrition level, and animal species, there are differences in follicular fluid compositions that either promote or impede oocyte maturation and further embryonic development (Mohammed et al. 2019). Leptin promotes the developmental competence of bubaline oocytes by modulating cumulus enabling factors and genes regulating pluries potency in the blastocyst (Gilchrist et al. 2016). Cumulus cells also play a crucial role in the substrate supply, transport, and production of chemical components for oocytes, such as microRNAs during oocyte maturation (Panda et al. 2017). Therefore, it may be concluded that the slicing method will yield more good quality cultivable buffalo oocytes than the aspiration method (Arul 2017).

The findings indicated that the buffalo oocyte collection method substantially impacted the number of oocytes produced. The aspiration plus slicing method was recommended for oocyte collection and showed larger COCs and a faster maturation rate.

CONCLUSION

The number, quality, and in vitro maturation of local buffalo were best suited by using oocytes that had been stored for 6 hours and cut into pieces and aspirated. Oocyte quantity was not significantly impacted by the ovary storage period, but quality and maturation rate were. Oocyte collection methods considerably impacted

oocyte number but had no discernible impact on oocyte quality or maturation rate.

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