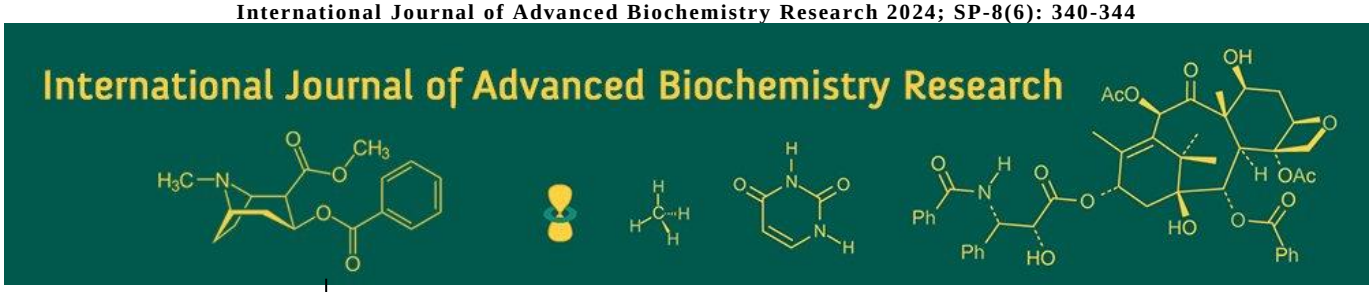

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Oocyte isolation techniques from the ovary samples of slaughtered animals: A review

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Abstract
The isolation of oocytes from the ovaries is the basic and essential step for *In vitro* Embryo Production. The oocyte recovery techniques differ between the live and slaughtered source of animals and the present review focuses on the recovery methods of abattoir ovary samples. The oocyte retrieval techniques include slicing, aspiration, puncture, dissection, and other techniques that are individually efficient in this process. The oocyte isolation techniques are associated only with the characteristics of quantity, and quality of oocytes and the characteristics directly influence the oocyte/ embryo developmental competence and gross IVEP. Based on the choice of laboratory and availability of Sample size the oocyte recovery methods are used and every oocyte isolation technique has attained its own pros and cons still there is no perfect technique has been invented in the reproductive field.

Keywords: IVEP, slaughtered samples, ovum, recovery methods

Introduction
Assisted Reproductive Technology (ART) like *In-Vitro* Embryo Production (IVEP) is a tedious process and influences several factors, including the oocyte collection technique. *In vitro* produced embryos have great demand in the market and are used for studies on IVM, IVF, IVC, commercial embryo transfer, embryo cloning, production of transgenic animals, and research purposes (Gordon I, 2003) [12]. Slaughtered ovary samples are cheap, abundant, reasonable, inexpensive, and various kinds of non-invasive oocyte isolation techniques are employed to collect oocytes from the ovary sample. The oocyte retrieval techniques include slicing, aspiration, puncture, dissection, and other techniques (Alm *et al*., 2008; Ciptadi *et al*., 2019) [2, 7].
Domestic animals play a vital role in livestock production and economics to contribute to the country's total GDP (Dash S, 2017) [9]. India is the world largest population country in the world and needs to produce a sufficient amount of food and food products from the livestock sector. So, genetically superior animal production and their maintenance which can be possible through IVEP is required. Different domestic animals include Cattle (Faheem *et al*., 2011) [11], Buffalos (Mishra *et al*., 2014) [25], Goats (Sigh *et al*., 2013) [37], Sheep (Davachi *et al*., 2012) [10], and Equines (Vazquez *et al*, 1993) [41] slaughter ovary samples are practically employed for the IVEP. In addition to ovum collection techniques, some other factors like breed, season, age, stage of the oestrus cycle, size of the ovary, type of the ovary, transportation time, and skill of the person influence the oocyte recovery rate (Wania *et al*., 1999) [46]. This review detailly addresses the different types of oocyte collection techniques for slaughter samples of domestic animals.

Oocyte collection techniques
Aspiration
Aspiration is an efficient technique to retrieve the A-grade oocytes from domestic animals like cattle, goats, and Sheep is experimentally proven and it yields the maximum number of oocytes for IVEP (Rakshitha *et al*., 2019; Jaayid *et al*., 2013; Wani *et al*., 1999) [31, 16, 46]. The advantages of this method is yielding high intact and unexpanded COC, less time for retrieval of oocytes, a minute amount of debris, less washing time of oocytes, and these all have an impact on the developmental competence of oocytes (Ahmed *et al*., 2015) [1].

The aspiration method not only recovers the maximum number of oocytes compared to the slicing technique and also influences the association of increases in the maturation rate of oocytes in goats (Majeed *et al*., 2011) [23]. Even though the recovery rate of oocytes is minimal in the aspiration technique compared with the slicing technique, the Advantage is this technique mainly has a chance of recovering a maximum number of good-quality oocytes (Rahman *et al*., 2016) [29]. The method of aspiration plus slicing is best among the oocyte collection methods because through aspiration technique can get the floating oocytes from the follicle and some follicles embedded in the deeper cortex have the oocytes recovered by the slicing technique (Hammad *et al*., 2014) [13]. Ultrasound-Guided Follicular Aspiration (UGFA) used to collect the oocytes from ovaries in live animals. The recovery percent and morphologically good quality of oocytes aspirated up to the maximum level by aspiration method from the ovaries of slaughtered animals (Majeed *et al*., 2019) [22], whereas the UGFA method is perfect for the higher developmental competence of *in vitro* culture embryos (Landeo *et al*., 2022) [20]. Quality oocytes (an ample amount of A and B-grade) are in most demand for *in-vitro* experiments and the aspiration technique is superior to producing the quality oocytes, but the oocyte retrieval is not only affected by various collection techniques but also influenced by the type of breed, reproductive status of animals, climatic conditions, poor body condition, and specimen processing factors (Raziq *et al*., 2008; Chaudhari *et al*., 2014) [32, 6]. The aspiration technique is good for the collection of A-grade quality oocytes within less time and debris.

Puncture

Puncture is a technique used to collect the oocytes from the slaughterhouse samples of ovaries. The Puncture method was applied to the abattoir ovaries of the Holstein cow results analysed that puncture is the perfect method to recover a higher number of good-quality oocytes and the oocyte developmental competence is not significant when compared to the other techniques (Wang *et al*., 2007a) [45]. The Slicing and Puncture techniques are associated isolation of A-grade (4-5 layers of cumulus cells and evenly distributed cytoplasm) cumulus oocytes complexes at maximum levels compared to Aspiration-1 and Aspiration-2 techniques, although the maturation rate of Boer goat is more than the slicing technique this is due to the recovery of the higher number of preantral oocytes from the ovaries (Wang *et al*., 2007) [44]. The sheep and goats are small ruminant domestic animals with small ovary size and Puncture is the best technique to collect the COC from the slaughtered animals (Bonde *et al*., 2000) [3]. Moreover, these techniques have a disadvantage in that recovery of more debris along with the oocytes and the techniques of Puncture, Slicing, or Aspiration don't affect the oocyte developmental competence during *in-vitro* studies (Hoque *et al*., 2011) [14]. If the slicing and puncture oocyte collection methods are taken, the highest COC may come from slicing, but the debris is much less in the puncture technique. Examining oocytes from the media is easy with this method, and oocyte maturation is more than slicing (Sofi *et al*., 2012) [38]. Among the aspiration, puncture, and slicing methods the puncture technique is associated with the collection of a greater number of good-quality due to the oocyte surrounding layers of cumulus cells stable and not disturbed at the time of retrieval and in the aspiration and slicing methods, it is opposite (Borah *et al*., 2018) [4]. Irrespective of quality the retrieval rate of oocyte yield is higher possibly by the slicing technique. Simultaneously, the majority of normal COC (A and B grade) production is associated with the puncture technique (Raihan Ratul, 2020) [30]. The Puncture or Slicing technique is prominently used for the retrieval of good-quality oocysts from the caprine slaughterhouse ovaries.

Slicing

The IVEP is important for the rearing of small ruminants and in which the basic oocyte isolation techniques like aspiration and slicing play a major role, especially in ovine seasonal breeders slicing enhances the recovery rate of oocytes in Ewe and Lambs (Shirazi *et al*., 2005) [35]. The comparison among the methods of slicing, aspiration, and puncture of oocyte retrieval concluded that slicing is the best technique for the isolation of oocytes and time-consuming processes associated with aspiration and puncture but, yielded a higher number of good-quality oocytes compared to puncture and aspiration techniques (Das *et al*., 1996; Nuaimi *et al*., 2020) [8, 27]. The slicing method dominates the other methods including puncture, aspiration, and aspiration after slicing of the oocyte isolation from the abattoir ovaries and not only affects the collection technique along with the transportation time from the slaughterhouse to the experimental lab but plays a vital role in the developmental competence of oocytes during *in vitro* conditions like prolonged time reduced oocyte competence and vice versa (W.M. Saleh, 2017) [43]. The goat ovaries are small and help properly handle oocyte collection by the slicing method. Employing aspiration and puncture or other techniques for oocyte isolation may be time-consuming and laborious (Pawshe *et al*., 1994) [28]. The ovarian stroma of buffalo species is strong and bottomless follicles are embedded in it so slicing is the method appropriate for the isolation of the oocytes even to get chance of debris like blood clots and fibrin shreds (Kumar and Mourya, 2000) [19]. A large number of primordial follicles and antral follicles are present in the prepubertal stage of buffalo and the slicing procedure to recover the deeper section of ovaries is one of the reasons to get a higher number of oocytes (Kumar *et al*., 1997) [18]. The recovery of oocytes is influenced by the size of the ovary and the mean recovery of oocytes per ovary is highest in the slicing technique in contrast with the aspiration (Sianturi *et al*., 2002) [36]. Even if the slicing method recovers a greater number of oocytes, eventually, the getting of quality oocytes is less due to the more debris associated with the oocytes and requires a long time to wash results the denudation of cumulus cells leading to less quantity of oocytes (Mahesh *et al*., 2014) [21]. The slicing method is associated with a high quantity of oocyte isolation and oocyte quality is not that much appropriate.

Dissection

Dissection is a simple technique to perform oocyte collection from the ovaries. The blunt dissection technique is easy to handle and an inexperienced person efficiently can collect the maximum number of oocytes (A and B grade) in contrast with the slicing technique from the goat abattoir ovary sample (Mamy *et al*., 2016) [24]. In addition, it yielded the maximum number of oocytes in contrast with the puncture and aspiration technique (Jamil *et al*., 2008) [17].

The dissection method is used to recover a good quality and quantity of oocytes from the slaughterhouse ovaries, especially bovines. The aspiration and dissection techniques are used for retrieving ovaries from domestic animals but dissection attained significant value due to the retrieval of more ovaries compared to the aspiration technique (Carolan *et al*., 1994) [5]. However, a minor difference was observed in the oocyte recovery processes between the dissection and aspiration techniques but the oocyte maturation rate in TCM-199 media is higher in case of the dissection technique (Hussain *et al*., 2015) [15]. The highest number of oocyte collection is associated with the dissection technique but the maximum number of normal oocyte collection is not (Tarikul Islam, 2019) [39]. The puncture and aspiration techniques are appropriate for the collection of oocytes from larger follicles visible on ovaries and the dissection method approaches the small, embedded follicles within the ovary. So, through the dissection method getting a high chance of quality oocytes.

Other techniques

The commonly employed techniques include slicing, aspiration, and puncture for slaughtered domestic animal oocyte retrieval for IVEP, and some other techniques are rarely used in labs. The involvement of these techniques are slicing plus aspiration in bovine (Udin *et al*., 2020) [40], follicular puncture plus slicing and scoring in buffalos (Varma *et al*., 2008; Shahid *et al*., 2014) [42, 33], slashing in Ewe Lambs (Shirazi *et al*., 2004) [34], and aspiration by a vacuum pump in camel (Nowshari, 2005) [26]. These techniques are a combination of two techniques or modifications of actual techniques and are not prominent in the practice of oocyte collection in *in-vitro* laboratories. As per the available review of the literature, these individual techniques are very efficient in the recovery of a bulk quantity of oocytes from slaughtered samples, even if these methods are not employed due to retrieval of a large number of debris, time-consuming process, and recovery of less quality of oocytes. So, each and every drawback effectively influences the oocyte developmental competence and further embryo developmental competence leads to a large impact on the IVEP.

Conclusion

There is no species-specific oocyte isolation technique or the most preferable ovum retrieval technique in the animal reproduction field and every technique has advantages and disadvantages. The oocyte retrieval techniques are influenced by several factors associated with oocyte number and morphology of oocyte but not early embryonic developmental competence. The availability of literature indicates that the slicing, dissection techniques are connected with the recovery of a higher number of oocytes, and the aspiration technique is related to the good quality of oocytes. Based on individual choice and circumstances of the laboratory oocyte isolation techniques are popular in the reproductive field.

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