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Factors influencing ovum pick-up technique results in cattle

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ABSTRACT

The Ovum Pick-Up (OPU) technique, which is used in in vitro embryo production (IVP) to retrieve immature oocytes from live donor animals, is one of the most important biotechnological procedures used in cattle breeding. The most important advantage of this technology is that it allows for the reproducible retrieval of immature oocytes from living donor animals. It is particularly useful in dairy cattle breeding to address infertility issues and boost the production of superior animals with high genetic value. The OPU technique offers several advantages, including its applicability to cows ranging from six-month-old calves to the first three months of pregnancy, its effectiveness in animals with genital tract infection or acyclic cattle, and its ability to yield a higher number of embryos within the same period compared to the Multiple Ovulation and Embryo Transfer (MOET) technique. Understanding and improving the technical and biological factors influencing the OPU procedure is necessary to increase and optimize donor animal use in IVP and the number of quality oocytes obtained. This review aims to examine the specifics of the OPU approach and the factors influencing its performance in light of contemporary literature, as well as to propose fresh ideas to researchers.

Keywords: bovine, in vitro embryo production, ovum pick-up, oocyte retrieval, follicle aspiration

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Introduction

Pieterse et al. (1988) pioneered the technique of transvaginal ultrasonography-guided follicular aspiration from live donor bovine ovaries in the late 1980s. This technique was initially used in cows who did not respond to superovulation (Kruip et al., 1994; Looney et al., 1994). The OPU technique aims to produce a large number of high-yielding calves while shortening the generation gap by using oocytes acquired repeatedly from the ovaries of heifers with high genetic value (Pieterse et al., 1991).

In 2020, in vitro embryo production (IVP) produced 79.7% of all embryos transplanted worldwide. Additionally, 71.2% of the embryos obtained from IVP were obtained with OPU. This increase in IVP is attributed to OPU (Viana, 2021 and 2022).

Two methods are primarily used as oocyte sources in in vitro embryo production. Transvaginal ultrasonography-guided follicular aspiration technique in live donor animals is one of these approaches, while postmortem follicular aspiration from slaughterhouse material is another (Galli et al., 2014; Ferré et al., 2019). OPU is a non-invasive and reproducible approach for retrieving large numbers of immature oocytes from the antral follicles of live donor animals (Choudhary et al., 2016; Watanabe et al., 2017; Çizmeci, 2022). OPU/IVP devices can extract 3-8 mm diameter follicles from the ovaries of calves, heifers, and even pregnant animals. The OPU procedure offers

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the only alternative for obtaining cumulus-oocyte complex (COC) from pregnant animals (Aller et al., 2012; Ferré et al., 2019). OPU does not disrupt the reproductive cycle of the donor. It can be performed successfully at any time, regardless of the donor's reproductive state. It can be repeated at regular intervals (every 1-2 weeks). It causes the least amount of stress for the donor animal. Additionally, each application has a reduced cost. An immense amount of oocytes can be fertilized in vitro (IVF) with a single straw spermatozoa. It allows the use of several premium bull semen and sexed spermatozoa. It enables dominant follicle ablation (DFR). With OPU, more transplantable embryos can be obtained from each donor monthly (Qi et al., 2013; Boni, 2012; Choudhary, 2016; Kimble, 2020). Unlike oocytes taken from ovaries collected at a slaughterhouse, the genetic characteristics and health state of the donor are known in OPU oocytes. Furthermore, the OPU procedure is a promising technique that can be included in programs for animal breeding that use endangered breeds (Borş et al., 2018).

Retrieving oocytes from the ovaries of donor animals by OPU technique has brought a new perspective to assisted reproductive techniques and made it possible to use donor animals with greater genetic potential for IVP for many years (Bols and Stout, 2018).

Factors influencing ovum pick-up technique results

The final outcome of the OPU technique is affected by two main factors: technical and biological factors. Technical factors influencing the success of the OPU technique include aspiration pressure, the type and frequency of the ultrasound device, aspiration needle characteristics, and operator experience. Biological factors influencing the success of OPU include individual differences among donors, breed, age, health status, body condition score (BCS) and nutrition, as well as aspiration frequency and superstimulation. In OPU programs, the generation of embryos also requires specialized laboratory support (Galli et al., 2014; Da Silva et al., 2016; López, 2020).

Aspiration pressure value

The aspiration pressure in the OPU mechanism depends on the aspiration pump, the length and diameter of the tube used, the size and type of the collection tube, and the diameter of the aspiration needle. By adjusting the aspiration needle's width, the rate of liquid aspiration can be increased up to three times while maintaining the same aspiration pump pressure. Although high aspiration pressure increases the number of oocytes collected, it is stated that high aspiration pressure (70-130 mm/Hg) reduces the rate of oocytes with cumulus cells. It is suggested that low aspiration pressure increases the rate of reaching the blastocyst by increasing the rate of cumulus cell oocytes (Bols et al., 1996; Fry et al., 1996; Ward et al., 2000; López, 2020). The value of the aspiration pressure may damage the cumulus-oocyte complex. Following follicle aspiration, the morphology of the COCs should be assessed when experimenting with various aspiration pressures and needle sizes. As a result, it is possible to identify the aspiration pressure range or threshold value that will limit harm to the aspirated COCs. This technique can be used in systems using disposable injection needles to calibrate in vitro (Bols et al., 1996).

Resolution of the ultrasound devices and features of the probes

Using ultrasonography, the follicles must be readily visible during the OPU procedure. The follicles are aspirated with the least harm to the donor and the COCs when clearly visible in the ovary. Research has shown that the ultrasonic screen's resolution and probe characteristics significantly impact the number of oocytes retrieved and minimize needless ovarian tissue damage (Bols et al., 2004; Çizmeci, 2022). For the operator to see the follicles and other ovarian structures in the ovary as clearly as possible, it is advised that high frequencies (6-8 MHz) be used (Da Silva et al. 2016; López 2020). New ultrasound devices even show follicles with tiny diameters (2-3 mm), which are not suitable for oocyte retrieval. Although sector probes are the most commonly used in OPU systems, linear probes can also be used. There is no statistical difference between sector and linear probes in terms of oocyte number and quality, although the sector probe visualizes tiny follicles better than the linear probe. The linear probe, on the other hand, restricts ovary movement to a rotation along its longitudinal axis and hence cannot observe the ovary's outside margins. (Bols et al., 2004; López, 2020).

Aspiration needle diameter and type

The sharp aspiration needle is the most essential technical aspect influencing a successful OPU application (Seneda et al., 2001; Singh et al., 2003; Bols et al., 2004). Previously, operators used needles that were 50-60 cm long and had an outside diameter of 1-1.5 mm (Looney et al., 1994). The main downside of these needles is that they lose their sharpness rapidly and cannot be re-sharpened to their previous sharpness. Furthermore, these long, non-disposable needles are rather costly. Alternative OPU systems that use disposable 18-gauge (1.02 mm) epidural needles or subcutaneous injection needles that are somewhat less expensive have been developed (Rath, 1993; Bols et al., 1995). These needles have the advantage of being sterile and available in various sizes and lengths (Bols and Stout, 2018).

It has been reported that using needles with a diameter of less than 18 g in OPU systems results in a higher number and quality of oocytes. The oocytes are separated from the surrounding cumulus cells because they move quicker than the cumulus cells due to the use of large-diameter needles. Furthermore, the use of large diameter needles causes more damage to the ovary. Damage causes an increase in blood aspiration. The use of needles with tiny diameters (more than 21 g) decreases the aspiration rate, increasing the amount of oocytes lost. As a result, 18-20 g aspiration needle sizes are recommended (Bols et al., 1996; Da Silva et al., 2016; López, 2020).

The optimal needle length is thought to be between 40 and 75 mm. Aspiration needles 40 mm long require extensive manipulation during follicular aspiration because they cannot reach all follicles from the perforated area of the ovary. When aspiration needles longer than 75 mm are used for follicular aspiration, they are flexible and easily bend (López, 2020).

The curve of the needle tip, on the other hand, influences the fraction of oocytes with compact cumulus cells. There are two types of needle tips available on the market: short and long conical, and it has been reported that using a long conical needle enhances oocyte yield (Bols, 1997).

OPU session application frequency

The OPU technique is highly reproducible (Pieterse et al., 1991; Watanabe et al., 2017). The frequency and duration of the OPU session used on the donor animal, on the other hand, influences the quality and number of oocytes retrieved (Nolan et al., 1998; Merton et al., 2003; McEvoy et al., 2006). Although a 3- or 4-day delay between two OPU treatments has been shown to give fewer COCs than a 7-day interval, a 3-day interval has also been shown to yield better quality COCs and a higher blastocyst rate. This is because the dominant follicle (DF), which arises roughly three days after the OPU treatment, suppresses the development of other follicles. The number of oocytes collected per session does not differ between the OPU program performed at 3 and 4-day intervals, which is often used, and the OPU program performed at 2 and 5-day intervals (Merton et al., 2003; Sirard, 2012; López, 2020).

Donor animal's diet

The donor's diet influences follicular development and ovulation through its role in the hypothalamus-pituitary-gonadal axis (Armstrong et al., 2003). Negative energy balance in cattle negatively affects pre-ovulatory follicle diameters and follicular development (Armstrong et al., 2001). Malnutrition has negative effects on the in vitro developmental competence and blastocyst rate of oocytes retrieved from the donor animal (Dominguez, 1995, Ruiz et al., 1996). Furthermore, a correlation has been noted between an increase in BCS and an increase in the developmental capacity of the oocyte (Dominguez, 1995). Conversely, low BCS in the donor animal due to malnutrition has a negative impact on the blastocyst rate and the ability of the resulting oocytes to grow in vitro (Bols and Stout, 2018; López, 2020). It has been noted that moderate to high BCS is linked to the negative effects of excessive calorie intake (Sartori et al., 2017). Furthermore, prolonged hyperinsulinemia decreases the ovaries' sensitivity to gonadotropins and oocyte quality, even though high energy intake raises blood sugar, insulin, and insulin-like growth factor-1 (IGF-1) concentrations (Diskin et al., 2003; Bender et al., 2014; Sales et al., 2015).

Donor animal species

Depending on the breed of the animal, the AFC in the ovaries of cattle receiving repeated OPU treatments differs (Goodhand et al., 1999; Viana et al., 2010). Bos taurus and Bos indicus cow breeds vary considerably in the number of follicular waves, the diameter of the DF, and the rate of follicle development (Figueiredo et al. 1997; Bó et al. 2003). The quantity of oocytes extracted from each OPU varies significantly between breeds, according to several research groups (Pontes et al., 2010; Gimenes et al., 2015). It has been noted that compared to cattle of the Bos taurus breed, the number of follicular waves and the AFC that occur during the follicular wave in Bos indicus breed cattle are higher. Oocytes from Bos indicus cow breeds are obtained in greater quantities as a result of this circumstance (Watanabe et al. 2017; Bó et al. 2019).

In cattle of the Bos taurus species, the average number of oocytes recovered per OPU treatment is 4–14, whereas in the cattle of the Bos indicus species, it is 18 –25 (Thibier, 2004; Rubin et al., 2005; Martins et al., 2007; Pontes et al., 2011). According to Sartori et al. (2010a), the average AFC in the ovaries of Bos taurus cattle is twice that of Bos indicus cattle. Breed-related variations in circulating insulin, IGF-1, and cholesterol are thought to be connected to this (Alvarez et al., 2000; Sartori et al., 2010b and 2018a).

Because of their larger AFC, superior oocyte quality, and greater sensitivity to gonadotropic hormones, Bos indicus breed cattle have substantially more economical IVP than Bos taurus breed cattle (Sartori et al., 2018b).

Donor animal age

Many eligible donor animals, ranging from 6-month-old calves to 3-month-old pregnant cows, can be used with the OPU approach (Aller et al., 2012; Ferré et al., 2019). Because the follicular reserve in the ovaries of five-year-old cows declines, the age of the cattle must be considered in IVP (Cushman et al., 2009). It is reported that the blastocyst rate of oocytes collected from donors aged one to three years is higher than that of older donors (Ali et al., 2021).

This is significant because genetic development can be expedited by retrieving oocytes from prepubertal calves and decreasing the intergenerational period (Landry et al., 2016; Çiftçi, 2022; Çizmeci, 2022). The developmental competence of oocytes retrieved from calves less than six months old, on the other hand, is poorer (Duby et al., 1996; Ax et al., 2005). It has been reported that the number of small to medium-sized follicles and the total number of follicles aspirated after ovarian stimulation are higher in young Bos taurus breed donors compared to cyclic donor cattle. Although in vitro maturation produces more COCs, young animals produce less blastocysts than cyclic cows (Landry et al., 2016; Baldassarre et al., 2018; Zacarias et al. 2018; Seneda et al., 2020). It has also been observed that IVP efficiency is higher in heifers of Holstein breed donors than in lactating cows (Ferreira et al. 2011; Ali et al., 2021).

The OPU procedure in calves is dependent on the pelvic size to accommodate the vaginal probe. Oocytes can be acquired with the OPU technique in Holstein breed heifers at the age of 6-9 months, depending on the size of the probe used (Bols, 1999; Bols and Stout, 2018).

Heat stress and season

Heat stress during follicle development in cattle has been shown to affect oocyte quality and embryo development by preventing follicular dominance (Qi et al., 2013). It is suggested that the negative effects of heat stress on fertility lower oocyte quality in a short period of time by causing problems such as the small diameter of the DF of follicular waves, inadequate dominance formation, and an increase in the number of large-diameter follicles (Çizmeci, 2022). Heat stress has been shown to reduce the developmental ability of oocytes retrieved from both OPU and slaughterhouse material. During the summer months, the quality of bovine oocytes decreases, and the lipid composition of oocyte membranes varies (Al-Katanani et al., 2002; Takuma et al., 2010; Boni, 2012; López, 2020). According to Roth et al. (2001), the quality of oocytes acquired at the beginning of autumn is low and gradually improves as winter approaches. Heat stress has been shown to disrupt the GnRH release mechanism from the hypothalamus, resulting in lower levels of FSH and LH in the bloodstream, which negatively affects follicle selection and development and reduces oocyte quality (De Rensis and Scaramuzzi, 2003; Camargo et al., 2006; De Rensis et al., 2021; Çizmeci et al., 2022).

Follicular wave synchronization

It has been shown that follicular development in the bovine ovary occurs in waves and that two or three follicular waves are often seen in a single estrous cycle. (Adams and Pierson, 1995; Adams, 1999; Sirard, 2018). On random days of the estrous cycle, follicles with a diameter of at least 2 mm are aspirated in several OPU/IVP protocols (Pontes et al., 2011; Dos Santos et al., 2016). Oocyte quality, oocyte growth rate, and IVP are all affected by the time of the estrous cycle during follicle aspiration (Seneda et al., 2001; Hendriksen et al., 2004; Camargo et al., 2006). It has been discovered that oocytes aspirated on the fourth, fourteenth, and eighteenth days after ovulation of the estrous cycle yield a greater rate of blastocysts (Gonįalves et al., 2022). It has been observed that more and higher quality oocytes are retrieved during the recruitment phase when the DF does not develop due to follicular atresia. It has also been reported that oocytes retrieved at this stage are more likely to reach the blastocyst (Bacelar et al., 2010; Gimenes et al., 2015; Ongaratto et al., 2015). The synchronization of the follicular wave in donor animals makes the follicles more homogenous in terms of diameter and developmental stage, allowing for the retrieval of higher-quality oocytes. Synchronizing the follicular wave before OPU has been shown to boost the number of embryos generated and pregnancy rates after the transfer. The estrous cycle of cattle and the physiology of the follicular wave ensure that follicular waves are synchronized before the OPU. As a result, follicular wave synchronization can be used to improve IVP findings prior to OPU (Cavalieri et al., 2018; Seneda et al., 2020). Follicular dynamics in the estrous cycle in cattle can be manipulated with exogenous gonadotropin applications (Aerts and Bols, 2010). A follicular wave has been observed in cattle approximately 36 hours following gonadotropin application (Bergfelt et al., 1994 and 1997; Ongaratto et al., 2015).

The goal of administering PGF2α four days before OPU is to ensure CL regression. In the absence of CL, follicular visibility and puncturing are easier, vascular perfusion reduces, and thus, the number of COCs collected increases (Bacelar et al., 2010; Ongaratto et al., 2015; da Silva et al., 2017). However, oocyte quality has been found to decline as a result of a decrease in plasma P4 concentration by inducing luteolysis prior to OPU (Nasser et al., 2011). It has been shown that adding P4 to the treatment protocol in dairy cows that receive superstimulation during the first follicular wave improves embryo quality (Rivera et al., 2011).

GnRH stimulation 48 hours before OPU in the early lactation phase improves embryo production efficiency in Holstein breed donors. Furthermore, it has been found that EB stimulation of donor cattle enhances the effectiveness of embryo formation in OPU-IVP technology. In cow studies, it has been shown that those who received EB application had higher levels of AFC and COCs than those who had GnRH stimulation (Ogata et al., 2015; Cavalieri et al., 2018; Hidaka et al., 2018).

Nonhormonal application and superstimulation

The OPU approach is a non-invasive, reproducible procedure that can be performed with or without hormonal stimulation (Watanabe et al., 2017; Wrenzycki, 2018). Once or twice a week, two distinct OPU procedures, with or without hormonal stimulation before OPU, might be applied (Chaubal et al., 2007). There is no stimulation in the standard OPU procedure, and OPU can be applied to each donor animal twice a week. It has been found that OPU application twice a week delivers the largest amount of oocytes of adequate quality when compared to OPU application once a week. This is because all observable follicles are aspirated, preventing the DF from growing and inhibiting the growth of other follicles. Thus, there is no need for ablation (DFR) of the DF. (Qi et al., 2013; Çizmeci 2022). OPU application twice a week can achieve 130 embryos every year, resulting in the delivery of 70 calves (Merton et al., 2003).

The second method is the application of hormones called "Superstimulation". The purpose of superstimulation is to increase the number of oocytes available for aspiration per OPU administration. The number of oocytes retrieved from superior cows can be enhanced by using exogenous gonadotropins and managing follicular dynamics. Equine chorionic gonadotropin (eCG), pituitary extracts from pigs and sheep, and human menopausal gonadotropin (hMG) are all used for superstimulation in cattle (Mapletoft et al., 2002; Aerts and Bols, 2010). Due to variations in properties such as half-lives and LH secretion, a variety of protocols have been developed for superstimulation with these hormones. Changes in the dosage and timing of gonadotropins are necessary because the primary goal of pre-OPU superstimulation is to generate more follicles rather than multiple ovulation (Bols and Stout, 2018). Numerous techniques have been tested for this aim, including lowering the amount of FSH and dissolving it in various polymers, using a single dosage of FSH in pre-OPU superstimulation protocols, and using different application routes (Chaubal et al., 2007; Ongaratto et al., 2010; Vieira et al., 2016). Applying exogenous FSH to donor cattle can enhance the quantity and quality of oocytes retrieved with OPU in cattle. This temporary increase in serum FSH concentration can delay the development of the DF, atresia of the subordinate follicles, and the increase in the size of the ovarian follicles, making them suitable for OPU (Ongaratto et al., 2015 and 2020; Fernandes et al., 2020; Çiftçi and Dinç, 2023). One of the major parameters for increasing the effectiveness of IVP systems is the diameter of the follicles in the ovary during OPU (Vassena et al., 2003). FSH treatment before OPU can improve the number of medium and big follicles as well as the effectiveness of OPU-IVP (Bó et al., 2019; de Carvalho et al., 2019; Ongaratto et al., 2020; Çiftçi and Dinç, 2023). However, the response to exogenous FSH can be affected by factors such as the change in the applied FSH concentration, breed, application method, application frequency, and the interaction of FSH with LH (Sartori et al., 2010a; Zacarias et al., 2018; Kaya et al., 2018; Fernandes et al., 2020). It is reported that FSH treatment and regimen, although it increases the number of medium-sized follicles (4-6 mm), has no effect on the quality and number of oocytes obtained. It has been shown that dividing the FSH regimen into 7 days rather than 4 reduces the number of small antral follicles (1-5 mm) while increasing the number of large antral follicles (>9 mm) (Mapletoft et al., 2015).

More AFC, COCs, and embryos can be obtained with superstimulation. Long-term exogenous hormone use, on the other hand, may alter the donor's hormonal condition and result in infertility. Additionally, the response of other donors to hormone stimulation may differ. As a result, it has been observed that short-term hormonal stimulation is beneficial (Qi et al., 2013; Vieira et al., 2014). In donors with low AFC, AFC can often be raised using FSH-LH combinations or eCG. These hormones are usually used in ET programs. Because the primary goal of pre-OPU superstimulation is to produce more follicles rather than to ensure multiple ovulation, the dose and timing of the treatment are critical (De Rover et al., 2008; Bols and Stout, 2018).

Because of the low number of oocytes retrieved each OPU session from Holstein cattle, follicular wave synchronization becomes necessary. As a result, it has been reported that FSH superstimulation is essential to increase embryo production (Demetrio et al., 2020). In Holstein breed donors without hormonal stimulation, 4-5 quality oocytes are retrieved each

OPU session, but up to 20 are obtained in those with hormonal stimulation (de Loos et al., 1989; Hasler, 1998; Bols et al., 2005; Vieira et al., 2014 and 2016). It has also been shown that the average number of aspirated follicles, COCs, and embryos is higher in superstimulated animals than in non-stimulated animals (Chaubal et al., 2007; De Roover et al., 2008).

Prolonged stimulation of the ovaries following a single eCG injection causes problems for practitioners all around the world (Bo and Mapletoft, 2014). The half-life of eCG in the cow is 40 hours, and it can stay in the bloodstream for up to 10 days (Murphy and Martinuk, 1991). Consequently, eCG's extended half-life results in persistent ovarian stimulation, non-ovulated follicles, abnormal endocrine profiles, and poor embryo quality (Kruip et al., 1984; Aller et al., 2012). However, Ribas et al. (2018) found that using 800 IU eCG before OPU increased the rate of follicles >6 mm in diameter and that the oocytes obtained from these follicles had a higher fertilization rate that supported the first embryonic development, and that this was an alternative protocol for ovarian superstimulation before OPU.

Coasting, or ovarian stimulation followed by a gonadotropin-free rest interval, has been proven to be a successful regimen for producing higher quality oocytes and improving blastocyst output in cattle. The coasting process is described as depriving cows of FSH for 36-48 hours prior to OPU. In other words, the time between the last FSH application and OPU is referred to as partial in vivo prematurity. Coasting is achievable in FSH-stimulated cows by ceasing FSH application 36-48 hours before OPU, but it is not possible in eCG-stimulated cows due to the extended half-life. Coasting in ovarian stimulation regimens has been shown to increase blastocyst output in cyclic cows by up to 80% (Blondin, 2002; Nivet et al., 2012; Landry et al., 2016; da Silva et al., 2017).

Dominant follicle ablation (DFR)

When a DF is absent or eliminated at the start of superstimulation, the overall number of oocytes and live embryos increases significantly when compared to the outcomes of superstimulation in the presence of a DF (Merton et al., 2003). The sensitivity of the ovaries to superstimulation differs greatly between donors (Looney, 1986). Ablation of the DF or hormonal treatments can be used to optimize the superstimulation response and increase the number of antral follicles. The DF in the ovary suppresses follicular development by stimulating the release of inhibin and estradiol (Aerts and Bols, 2010). It has been observed that the presence of a DF lowers the in vitro developmental competency of oocytes produced from subordinate follicles (Hendriksen et al., 2004). DFR is typically applied 48 hours before the OPU session. DFR causes an increase in FSH to begin within 12 hours and a new follicular wave to start within 24 hours. It is also claimed that DFR is as effective as protocols combining progesterone and estradiol in follicular wave synchronization for superstimulation in cattle (Bó and Mapletoft, 2014; Adams and Singh, 2021; Çizmeci 2022).

Reproductive health of the donor animal

OPU induces both short- and long-term alterations in donors' ovaries and vagina. The vaginal fornix can be perforated up to 48-72 hours after puncture (Da Silva et al., 2016). According to one study, although bruising was found in the perivaginal area, this did not cause significant harm to the donor (Viana et al., 2003). It has also been noted that needle puncture can cause vaginal tears or pathogen contamination (Younis et al., 1997; Cho et al., 2004). Adhesions and fibrosis can be seen in cow ovaries, particularly those subjected to conventional OPU treatments over an extended period (Da Silva et al., 2016). The frequency and high number of repeated OPU sessions result in the formation of an abnormally large amount of fibrous connective tissue in the tunica albuginea and ovarian stroma (Viana et al., 2003; McEvoy et al., 2006; Çizmeci, 2022). Many researchers, however, have reported that ovarian tissue is resistant to sclerosis. It has been reported that cattle can tolerate the follicular puncture process, which disrupts ovarian surfaces and significantly changes tissue dynamics (McEvoy et al., 2002; Bogh et al., 2003). The OPU technique can be applied to the same donor animals for 4-5 months without significant complications (Kruip et al., 1994; Petyim et al., 2007; López, 2020). In the ovaries of donor animals treated with OPU, connective tissue formation, inflammatory cell infiltration, and the presence of luteal tissue scattered in the stroma may occur (Viana et al. 2003; López 2020).

The use of epidural anesthetic on donor animals is one of the concerns that must be considered in OPU operations to ensure that the follicular aspiration process is carried out in a healthy manner and to prevent the entry of pathogens. Epidural anesthesia is the treatment that causes the most discomfort to donor animals during OPU application (Petyim et al., 2007). Epidural anesthesia reduces the donor animal's disturbing movements and abdominal tension. Repeated OPU applications show that lasting changes may develop in the epidural anesthesia area in cattle. Animal welfare, problems of epidural anesthesia, disruption of the integrity of the ovarian stroma, and adhesions should all be considered in aggressive OPU administration twice a week (McEvoy et al., 2006; Chaubal et al., 2007; Çizmeci, 2022).

Conclusion

As a result, the OPU technique is frequently used to retrieve oocytes in IVP, both in commercial enterprises and embryo transfer research. In comparison to MOET, IVP has become a better commercial choice because it produces more embryos in the same time frame as OPU, permits the use of different elite bull sperm in IVF, and does not require the use of medications. For this reason, veterinarians and researchers intending to use the OPU option should familiarize themselves with the intricacies of this technique and the factors influencing its success. This knowledge ensures a more extensive use of donor animals and the acquisition of higher quality oocytes.

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