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Importance of animal welfare in sheep reproductive technologies

Az állatjólét fontossága a szaporodásbiológiai technológiák alkalmazása során juhokban

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ABSTRACT

Reproductive performance of sheep has a decisive impact on the productivity of the flock, but this is influenced by many factors (e.g. stress, management technology). Applying assisted reproductive technologies ensures genetic selection and genetic progress in sheep farming. However, the application of these methods also has an impact on animal welfare. Animals need to experience as little stress and pain as possible during reproductive and animal health interventions, which can be achieved by optimising husbandry technology and continuously monitoring the stress levels or pain of the animals. Several methods can measure the level of stress and pain induced by various interventions, one of the most important is measuring cortisol concentration (from blood, saliva, wool, urine or faeces), since in the event of a negative stress effect, the hypothalamic-pituitary-adrenal axis is activated and the concentration of cortisol produced thus shows the degree of stress. Cortisol also has a negative impact on reproductive performance. In addition, the well-being of animals can be monitored using other precision devices, like boluses and accelerometers. During assisted reproductive interventions, the goal is to compare different techniques and then choose the one that causes minimal stress and pain for the animals.

Keywords: assisted reproduction, cortisol, sheep, stress, well-being

ÖSSZEFOGLALÓ

A juhok reprodukciós teljesítménye döntő hatással bír az állomány termelékenységére, azonban ezt számos tényező befolyásolja (pl.: stressz, tartástechnológia). Az asszisztált reprodukciós technológiák alkalmazása biztosítja a genetikai szelekciót és a genetikai előrehaladást a juhászatokban. Azonban ezen technológiák alkalmazása hatással van az állatjólétre is. Fontos, hogy a szaporodásbiológiai beavatkozások során minél kevesebb stressz és fájdalom érje az állatokat, amely a tartástechnológia optimalizálásával és a juhok stressz szintjének vagy fájdalmának folyamatos monitorozásával érhetőek el. A különböző beavatkozások által kiváltott stressz és fájdalom szintje többféle módszerrel mérhető, az egyik legfontosabb a kortizol koncentráció mérése (vérből, nyálból, gyapjúból, vizeletből vagy bélsárból), mivel negatív stressz hatás esetén aktiválódik a hipotalamusz-hipofízis-mellékvese tengely és az így termelődött kortizol mennyisége megmutatja a stressz mértékét. A kortizol negatív hatással van a reproduktív teljesítményre is. Emellett más precíziós eszközök (bolusok vagy pedométerek) segítségével is monitorozható az állatok jólléte. Az asszisztált reprodukciós beavatkozások során a cél, hogy a különböző technikák összehasonlítását követően azt válasszuk, amely a lehető legkevesebb stresszel és fájdalommal jár a juhok számára.

Kulcsszavak: asszisztált reprodukció, állatjólét, juh, kortizol, stressz

INTRODUCTION

Assisted reproductive technologies (ART) are widely used to enhance reproductive performance and genetic improvement in modern sheep breeding (Amiridis and Cseh, 2012). The ART include oestrus synchronisation, superovulation treatment, artificial insemination, multiple ovulation and embryo transfer (MOET) (Callesen et al., 2019). Artificial insemination (AI) and MOET are used in cattle, swine and goat breeding, but the penetration of these techniques in the sheep industry is slower (Candappa and Bartlewski, 2011). Among the advantages of ART are the increasing genetic improvement, the gene preservation of endangered breeds, species, or animals with high value, and the reduction of the possibility of disease transmission (Cseh et al., 2012). In males, the reproductive efficiency can be increased through AI; in females, more embryos can be produced with MOET than in a natural breeding season (Candappa and Bartlewski, 2011; Vass et al., 2019). Despite the advantages and possibilities of the use of ART, it is crucial to consider the degree of stress caused by various interventions and to choose the least invasive and painful procedures. It is important to take into consideration the animal welfare and improve the efficiency of these procedures, which outcome of which cannot always be predictable.

THE MOET PROGRAMS IN SHEEP

There are several possible, more or less invasive ways to perform assisted reproductive techniques in small ruminants. For a MOET program, the donor and the recipient are synchronised, mainly with progestogen-containing devices (vaginal sponge or CIDR) (Gonzales-Bulnes et al., 2020). The superovulation treatment usually contains 200 mg FSH (Follicle-Stimulating Hormone) injection (generally distributed in six doses) and a single GnRH (gonadotropin-releasing hormone) agonist intramuscular injection, which can raise the number of viable transferable embryos (Menchaca et al., 2009; Figueira et al., 2020). Fertilisation can be done by natural mating or AI. In MOET programs, practitioners prefer to use AI with fresh or frozen sperm (Halbert et al., 1990). The most successful way of using the frozen sperm in sheep is laparoscopic insemination, where the thawed sperm is injected into the uterine horns (Gibbons and Cueto, 2011; Gebrehiwot et al., 2018). After 7-8 days from the AI or mating, the embryos are recovered from the donors in the morula or blastocyst stage (Naitana and Ledda, 2020). Generally, embryo collection is performed using a surgical technique under general anaesthesia (Callesen et al., 2019). In the case of laparotomy, the surgeon makes an approximately 7 cm skin incision cranial to the mammary gland. The uterine horns are externalised and flushed with embryo flushing medium. Then, the uterine horns are repositioned into the abdominal cavity, and the incision is sutured in multiple layers (Bergstein-Galan et al., 2017). Besides this, there are also less invasive methods that exist. One of them is the semi-laparoscopic embryo transfer, where the uterine horn is clamped with an atraumatic device guided by laparoscopy and pulled out from the abdomen through a small incision (Bari et al., 1999). The laparoscopic technique is a minimally invasive way to collect embryos, which can reduce post-operative adhesion but requires surgical skills. During the laparoscopic technique, three small (1-2 cm) incisions are necessary for the laparoscopic optic, the grasping forceps and the Foley catheter to perform the embryo collection in the abdominal cavity (McKelvey et al., 1986). Transcervical embryo collection is a non-surgical technique, but this method requires cervical relaxation (because the cervix is closed and difficult to penetrate in sheep) using hormones, either intramuscular (estradiol benzoate), latero-vulvar (cloprostenol) and intravenous (oxytocin) routes (e.g. Embrapa's protocol). The non-surgical technique can avoid general anaesthesia, intra-abdominal adhesions, and fasting. However, the transcervical technique is not widely used because of penetration difficulties (McKelvey, 1999; Fonseca et al., 2016). After collection and quality control of the embryos, they are transferred to recipients immediately or frozen in liquid nitrogen (Gibbons and Cueto, 2011). The embryos yielded by all the mentioned techniques can be transferred to the ipsilateral uterine horn to the corpus luteum (Cseh et al., 1991). All four techniques above can be used for ET but nowadays, minimally invasive techniques (laparoscopic or semi-laparoscopic) or non-surgical techniques are used mostly compared to surgical embryo transfer to minimise intraoperative and postoperative complications (Schiewe et al., 1984; McMillan and Hall, 1994; Candappa and Bartlewski, 2014; Gebrehiwot et al., 2018).

REPRODUCTIVE EFFICIENCY AND STRESS FACTORS

Animal welfare issues play a major role in small ruminant breeding and reproduction management (Roger, 2012). The development of agriculture and the new breeding technologies impacts the welfare of the animals. These new technologies (such as ovum and embryo transplantation) can cause distress or pain to the animals, so it is important to deal with animal welfare to reduce these negative effects (Clark et al., 2006).

Several stress factors (such as poor body condition, heat stress, handling procedures, and nutritional disorders) can decrease the reproductive performance of a flock by suppressing the ewes' normal oestrus behaviour and fertility (Dobson et al., 2012). Stress has an aversive effect on the maintenance of homeostasis. The hypothalamic-pituitary-adrenal (HPA) axis is vital in stress regulation. When responding to a stress stimulus, the corticotropin-releasing factor (CRF) is secreted from the hypothalamus, increasing the adrenocorticotropic hormone (ACTH) secretion in the anterior pituitary gland. The ACTH induces the secretion of glucocorticoids in the adrenal cortex (Smith and Vale, 2006). In females, another mechanism, the hypothalamic-pituitary-ovarian (HPO) axis, controls the female reproductive function. The gonadotropin-releasing hormone (GnRH) is produced in the hypothalamus and stimulates the adenohypophysis. In response to the stimulation, two hormones, the follicle-stimulating hormone (FSH) and the luteinising hormone (LH), are secreted and enter the bloodstream. The oestradiol and the progesterone are produced in the ovaries, and these hormones have a negative feedback mechanism on FSH and LH production. In the follicular phase, the concentration of oestradiol and the pulse frequency and amplitude of GnRH and LH define the follicular growth, the ovulation and the oestrus behaviour. The HPA axis inhibits the reproductive system during stress by suppressing the HPO axis function. Among other effects, the CRH inhibits GnRH secretion while cortisol inhibits the secretion of GnRH, LH, oestradiol and progesterone (Chrousos et al., 1998). Due to the inadequate GnRH/LH pulse frequency and amplitude, the follicular growth is incomplete with no ovulation, and the luteinisation fails (Dobson and Smith, 2000; Smith et al., 2003). When cortisol hormone was given in infusion from five days before the progesterone source was removed to five days after pessary removal, only one out of eight showed follicular activity and preovulatory LH surge. If cortisol treatment was given for five days after pessary removal, the oestradiol concentration remained low, and neither had an LH surge. When the ewes got cortisol for five days before pessary removal, surge-like secretion of LH was measurable in four ewes (out of seven) in five days after the progesterone source was removed (Macfarlane et al., 2000).

PRECISION LIVESTOCK FARMING TECHNOLOGIES IN REPRODUCTION

Modern technological developments are gaining space in agriculture to help farmers by monitoring animal health and welfare with sensors, boluses, microphones, and cameras (Schillings et al., 2021). These precision livestock farming (PLF) technologies help detect oestrus or parturition and, thus, improve reproductive indicators, mainly in the dairy industry so far. Accelerometer-type sensors incorporated into collars, pedometers, ear tags, or boluses are most commonly used for oestrus detection by noticing the activity changes on the oestrus day. Rumination time monitoring can also inform the farmers about the upcoming oestrus since the rumination time is decreasing due to the increasing locomotor activity (Das et al., 2023). Other non-invasive oestrus detection methods are measuring milk yield, temperature, and progesterone level. Furthermore, changes in skin temperature can also predict the upcoming oestrus. For calving prediction, intra-vaginal boluses can detect changes in behaviour and body temperature. Also, a belt bound to the animal recording the electrogastrogram is used to detect the uterus contractions (Mottram, 2016; Silva et al., 2021). Keeping the cows' and sheep's health under monitoring is challenging when the animals are on a large pasture. GPS-based collars help to determine the positions of the animals and their movements and can be used in combination with other sensors to evaluate animal welfare. Also, drones with cameras can record the animals' position and behaviour on the pasture (Herlin et al., 2021). Although PLF technologies are primarily used in the dairy cattle industry, some techniques have been developed in extensive dairy sheep farming in European countries (France, Italy, Greece, and Spain). These include electronic identification systems (ear tags, ruminal boluses, subcutaneous electronic devices), on-animal sensors (pedometer, rumination detectors), and automatic drafters or walk-over-weights (Vaintrub et al., 2021). To study the stress responses for the milking process in cows, the heart rate and heart rate variability (HRV) were measured with a Polar mobile recording system fastened to the animal's chest (Kovács et al., 2019). Accelerometers can explore the poor animal health or welfare conditions associated with lameness. The walking, standing and lying behaviour were changed between the lame and non-lame sheep (Kaler et al., 2020). As a pain-related behaviour, cows spent less time ingesting and ruminating and more time standing up without other activities for the systemic inflammatory challenge (Ledoux et al., 2023).

MONITORING STRESS LEVELS IN SHEEP

Behaviour changes

Due to a painful condition, the behaviour of sheep changes as they reduce their feed intake, lag behind the flock, show abnormal vocalisation and locomotion, lower their head, etc. (Steagall et al., 2021). The ethogram can be used to assess the effects of acute pain after laparoscopic soft tissue surgery in sheep. The Unesp-Botucatu sheep acute composite pain scale (USAPS) is a reliable, valid and sensitive scale to evaluate postoperative pain (Silva et al., 2020). The Sheep Grimace Scale can also assess the signs of pain and animal welfare conditions during a surgical intervention. The scale helps to evaluate pain management by analysing the facial expressions of the sheep. The orbital tightening, ear and head position, and Flehmen reaction show us the presence and degree of pain (Häger et al., 2017).

In addition to behavioural observations, we can measure the physiological parameters and blood biomarkers related to inflammation and stress factors. Measuring body temperature can be a good indicator of stress or health (Blanche and Maloney, 2017). Stress and pain can affect the heart rate, respiratory rate or even abdominal digestive motility (Raekallio et al., 1997; Dijkstra et al., 2018).

Cortisol level and other blood parameters

Cortisol is a widely used measure of stress in animals. Samples are collected by venipuncture (jugular vein) to measure the plasma cortisol, and for saliva cortisol, a synthetic swab is used in the animal's mouth. Saliva sampling causes less stress to the sheep than venipuncture, and animals should be habituated to blood sampling (Andanson et al., 2020). Another non-invasive method for measuring cortisol is the urine, faecal or wool cortisol concentration examination. From faecal samples, it is possible to detect the transport or novel environment stress in cows by measuring the cortisol metabolites (Palme and Möstl, 1997; Möstl et al., 2002). The urine cortisol concentration usually shows a chronic stress level in the animals (Asano et al., 2021). Under heat stress and water restriction conditions, measuring the wool cortisol concentration is a reliable method to evaluate the stress status of the sheep (Ghassemi Nejad et al., 2014). Besides cortisol, other biomarkers can also be measured from saliva samples. Among the lipase, butyrylcholinesterase, total esterase and adenosine deaminase measured from saliva during stress stimuli, lipase seems to be a biomarker of stress in sheep (Contreras-Aguilar et al., 2019). Even the daily handling procedures, such as isolation, tying and shearing, cause changes in blood biochemistry and the concentration of hormones. Plasma glucose, potassium, magnesium and cortisol levels increased, while sodium levels decreased after isolation, tying, or shearing (Carcangiu et al., 2008). Systemic proteins such as total plasma protein (TP) or acute phase proteins (APP) can be used as stress and inflammation markers. Inflammation or dehydration causes increased TP levels. The concentrations of positive APPs, such as haptoglobin and serum amyloid A, increase, while negative APPs (e.g. albumin, paraoxonase 1) decrease due to stress, trauma or injury (Costa et al., 2018).

ANIMAL WELFARE IN ART

Stress caused by isolation and fasting

Isolation is necessary during ART to monitor the effects of different techniques on the ewes. Due to the sheep being gregarious, the isolation can cause stress to them. If a group of sheep was isolated from the others in another pen for 24 hours, the serum cortisol concentration was elevated significantly, especially in the first hour. After that, the cortisol concentration showed a slow decrease for 24 hours, but it remained slightly increased than the control group. The change in plasma glucose level showed a similar pattern to the plasma cortisol concentration (Pierzchała et al., 1985). During the isolation, the animal's behaviour changed when the sheep were separated from the flock. The sheep spent more time in standing posture and vocalisation but less with lying, eating and ruminating than the control group. The plasma cortisol concentration in the isolation group was significantly higher one hour after the start of the separation, but 24 hours later, there was no significant difference compared to the control group. If the isolation is repeated, the sheep can become habituated to the situation (Cockram et al., 1994). In another study, the cortisol level elevation was also detected in the stress caused by the isolation. However, these results showed that the sheep cannot adapt to the isolation stress (Niezgoda et al., 1987). Dairy ewes after isolation stress were divided into two groups depending on the plasma cortisol concentration (high and low cortisol groups). After isolation, in the low cortisol group, the milking production was higher, and the somatic cell count was lower in the milk, compared to ewes with higher cortisol, so the cortisol level can affect the immune competence in ewes (Caroprese et al., 2010).

Other stress factors, such as shearing or fasting, can impact the oestrus behaviour or ovulation. Following a progesterone treatment, the second oestrus cycle was suppressed by shearing (seven days before the expected date of the oestrus) or fasting (also started seven days before the second oestrus). In these experimental groups, significantly more ewes showed a lack of oestrus behaviour than the control group. Some ewes in the treatment groups observed only follicular activity but no ovulation (MacKenzie et al., 1975). In estradiol-treated ovariectomised goats, three days of fasting can suppress the LH pulse frequency in light body weight animals (Tanaka et al., 2002).

Stress caused by different ART

When performing any kind of assisted reproductive technologies, we have to keep the animal welfare aspects in mind. It is necessary to minimise the stress, pain, suffering and the chance of infectious diseases (Muhammad et al., 2022). ART can cause acute or chronic pain in animals, so we have to investigate the degree of pain caused by the technology and choose the least invasive and painful method or pay attention to the appropriate anaesthesia and pain relief (Napolitano et al., 2020). In addition to its animal welfare aspects, stress can influence reproductive performance, as it was mentioned earlier. The stress during transport and handling reduces the LH pulse, frequency, and GnRH pulsatility, resulting in a failed ovulation (Smith and Dobson, 2002). A good reproductive performance does not always mean a good welfare state, but usually, behind poor fertility, we find illnesses, such as ketosis, locomotor disorders, and metritis (Ritter et al., 2019).

Cervical artificial insemination influences the plasma cortisol and oxytocin concentration. The cortisol concentration was higher in the plasma 30 minutes after the insemination procedure, even if it was easy or difficult to find the cervical entrance with an intravaginal speculum. The plasma oxytocin concentration increased 5 minutes after a difficult AI but then returned to the base level (Houdeau et al., 2002). Investigating the effect of repeated laparoscopy on plasma cortisol level, LH surge and ovulation in ewes, a study found that repeated laparoscopy procedures caused a sustained rise in plasma cortisol. Furthermore, ewes that underwent repeated laparoscopic procedures showed materially lower ovulation rates than ewes with one or two laparoscopic experiments (Martin et al., 1981). Comparing the heart rate and cortisol changes when the sheep were placed in a laparoscopy cradle or inseminated intrauterine by laparoscopy or cervically in a standing position, it was found that all of these procedures caused stress to the animals. The heart rate response was higher in ewes inseminated cervically than in sheep inseminated by laparoscopy. The cortisol concentration was elevated in all treated groups than in the control (non-treated) animals. The results showed that the laparoscopic intrauterine insemination did not cause higher stress than inseminating the ewes cervically in a standing position or placing them into the laparoscopy cradle (Khalid et al., 1998). Another study showed that laparoscopic AI causes stress to animals, but mostly because of the necessary restraints. The cortisol response could be reduced with diazepam treatment before the procedure, but it did not have the same effect on the heart rate (Haresign et al., 1995). The surgical (LP) and the transcervical (TC) techniques were also used successfully for embryo collection. Studying the inflammatory markers (total protein, haptoglobin, fibrinogen, paraoxonase-1) during these two procedures showed that they caused temporal effects on the inflammatory parameters, and there was no significant difference between the two methods. The haptoglobin (as a positive acute phase protein) concentration was elevated after the procedures, and the total protein increased nine days after the embryo collections. Depending on this result, even if the transcervical technique is less invasive than the surgical method, both cause alterations in the blood inflammatory markers (Oliveira et al., 2018). Investigating the stress response for the transcervical and surgical embryo collection showed that both procedures affected the ewe's welfare. Rectal temperature was lower during the laparotomy because of the anaesthesia. The heart rate was higher after the LP procedure than after the TC method. The LP embryo collection induced higher serum cortisol concentration, which was reduced three hours after the procedure. Furthermore, the cortisol concentration reduced slowly in TC because of the negative effect of cervical manipulation. Although the transcervical technique is faster and less invasive, success depends on the breed and the anatomical structures of the cervix, and attention should be paid to pain relief (Santos et al., 2020). In MOET programs, farmers can reduce costs and undue stress if they check the number of corpora lutea by laparoscopy to evaluate the response of the superovulation treatment before doing a surgical embryo collection (Bruno-Galarraga et al., 2015). According to the most recent (2023) FAO recommendation for embryo collection and transfer in sheep, the surgical technique is to be chosen because of the difficulties of the transcervical method. For the above reasons, laparoscopic intrauterine insemination can also be used instead of the transcervical technique (Blesbois et al., 2023).

Anaesthesia and pain relief during ART

Laparoscopy is a less invasive technique, but it causes pain to the animal, so it is important to find methods that can alleviate the pain. If only acepromazine (ACP) were used for sedation, the plasma cortisol concentration would be higher in ewes than in ewes sedated with acepromazine and ketoprofen combination or detomidine. Using acepromazine with ketoprofen increases the plasma cortisol concentration more quickly than using only ACP, so ketoprofen can reduce the duration of the stress period. Detomidine treatment before the laparoscopic surgery can eliminate the plasma cortisol rise because of its sedative and analgetic effect (Stafford et al., 2006). The α2-agonists (xylazine, detomidine, medetomidine, dexmedetomidine) could reduce the cortisol concentration after the embryo transfer in the goat and increase the pregnancy rate compared to the control group. However, there was no significant difference in the effectiveness among the different α2-adrenergic agonists (Aghamiri et al., 2022). Flunixin meglumine and dipyrone have analgetic effects, so these can be used after surgical procedures for pain relief. Using flunixin and dipyrone together can reduce the cortisol increase after surgical embryo collection because of the anti-inflammatory and pain-relieving effect (Balistrieri et al., 2023). However, during non-surgical embryo recovery, meloxicam was not effective in reducing pain and stress; meloxicam associated with dipyrone had a slight effect in reducing cortisol concentration (Ribeiro et al., 2023). After surgery that causes pain, the ß-endorphin concentration rises in the blood as an analgesic. Increasing the ß-endorphin level before a minor surgery did not have enough analgesic effect or was not long-lasting enough to prevent the aversive behaviour of the human handler (Shutt et al., 1989). Using any painful or stressful technique, we have to provide pain relief. Opioids have a great visceral pain relief effect, but the disadvantage is that this effect is short-lived in sheep. NSAIDs have good long-acting analgesic effects, so they are usable for reducing post-operative pain. Local anaesthetics (e.g. lidocaine) are used for infiltrating the surgical area or doing an epidural anaesthesia (Galatos, 2011). During the transcervical artificial insemination, the effect of the subarachnoid ketamine anaesthesia was examined. The result was that the ketamine reduced the pain (less vocalisation, abdominal contraction and normal ear movement), improved the pregnancy rate and made the cervical passage easier than the control group (Carnerio et al., 2019).

PROPOSAL FOR FUTURE RESEARCH

Due to the rapid increase in the population of the earth and food problems, new solutions are needed to improve the productivity of animal husbandry and reproduction. Utilising and improving assisted reproductive technologies in the small ruminant industry can be a key to development. Animal welfare and health are of common interest to researchers, farmers, and citizens. Considering that even the basic handling procedures can cause animal stress when applying these reproductive technologies, we must pay serious attention to the animal's stress state. The least invasive technology available should be chosen to cause minimal animal pain and stress. To evaluate animal welfare, precision devices need to be developed in the small ruminant industry because it is not as widely used as in cattle. The appropriate anaesthesia and pain relief can decrease the pain and accelerate regeneration during the procedures. Further studies are necessary to improve the knowledge about the stress level caused by different ART to choose the gentlest and successful reproduction method that can also maintain efficiency.

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