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Use of laparoscopy in assisted reproduction of small ruminant

T. DOVENSKI¹, P. TROJAČANEC¹, V. PETKOV¹, LJ. KOČOSKI¹ and J. GRIZELJ²

Faculty of Veterinary Medicine, Ss. Cyril and Methodius University, Skopje, R. Macedonia

¹ Faculty of Biotechnical sciences, University St. Kliment Ohridski- Bitola, R. Macedonia

² Faculty of Veterinary Medicine, University of Zagreb, R. Croatia

Abstract

The assisted reproductive technologies are used to accelerate genetic gain and improve reproductive performances in farm animals, including small ruminants. This technologies includes estrous synchronization, artificial insemination (AI) using fresh, frozen or sexed semen, embryo transfer (ET) using *in vivo* or *in vitro* produced embryos, and more advanced - cloning using somatic cell nuclear transfer procedures and production of transgenic animals. Various equipment and diagnostic procedures, such as ultrasonography and laparoscopy, have been used as additional tools for monitoring the ovarian response to superovulatory treatment in donor animals as well as for less invasive collection and transfer of gametes and embryos.

The aim of this article is to review the status of implementation of laparoscopy in Assisted Reproduction Technologies (ART) of small ruminants and to present our experience in this field.

Since 1968, when Roberts reports on the first endoscopic examination of reproductive tract in sheep, laparoscopy became an important tool for studying reproductive physiology. Initially, as a technique for direct observation of ovaries and uterus, it has been used for study of ovulation, follicles and corpora lutea development, as well as for pregnancy diagnosis. Corresponding with the rapid development of assisted reproductive methods, laparoscopy has been introduced as a technique for enhancing its results. Salamon and Maxwell (2000) quoted "...of the several methods used to improve the fertility after insemination, the most effective was laparoscopic intrauterine insemination". The problem of the "cervical barrier" has been overcome and satisfactory fertility rate has been achieved by significant reduction in the number of spermatozoa per insemination (from 200-300 to 1-10 millions, or less for sex-sorted semen). Pregnancy rate is commonly over 75% and 50% for goats and sheep respectively.

Laparoscopic technique has been introduced in MOET programs by McKelvey et al (1986). Primary purpose was to reduce adhesions of the reproductive system after surgical embryo recovery.

Laparoscopy enables repeated flushing of sheep and goats uterus and satisfactory embryos recovery rate. Also, fresh or frozen-thawed embryos are being transferred to the utera or oviducts of recipients by laparoscopy, achieving high pregnancy rates. It is recommended by many authors as a minimally invasive surgical procedure. Laparoscopy is also used for oocytes aspiration (Laparoscopy Ovum Pick Up - LOPU) in small ruminants.

The use of laparoscopy for assisted reproduction techniques in Macedonia begun in early 90's by purchase of the first set of „Karl Storz“ equipment. After an initial period of several months for adopting the standard routine we have concluded several scientific projects where laparoscopy was used for intrauterine inseminations as well as for recovery and transfer of embryos in sheep and goats. Later we tried to introduce laparoscopic inseminations in the routine farm practice. Ovine intrauterine/intracornual insemination by frozen-thawed semen has resulted with pregnancy rates from 45% to 60%, when AI was performed out of season and during the breeding season, respectively. In goats this percent sometimes was even higher (over 80%).

In conclusion, laparoscopy can be useful additional tool in ART of small ruminants, although this method is limited for implementation in routine practice, since it requires expertise and higher expenses.

Laparoskopija u postupcima asistirane reprodukcije malih preživača

Sažetak

Postupci asistirane reprodukcije koriste se u svrhu ubrzanja genetskog progresa i poboljšanja reproduktivnih svojstava u farmskih životinja, uključujući male preživače. Ovi postupci uključuju sinkronizaciju estrusa, umjetno osjemenjivanje (UO) svježim, smrznutim ili seksiranim sjemom, embriotransfer (ET) *in vivo* i *in vitro* proizvedenih

zametaka te napredne tehnike – kloniranje nuklearnog transferom podrijetlom iz somatskih stanica i proizvodnja transgenetskih životinja.

Različita oprema i dijagnostički postupci, poput ultrazvuka i laparoskopije, dodatni su alati koji se koriste za praćenje jajničkog odgovora pri superovulatornom postupku davateljica kao i za manje i invazivne postupke dobivanja gameta ili zametaka i njihovog transfera.

Cilj ovog rada je prikaz korištenja laparoskopije u postupcima asistirane reprodukcije (PAR) malih preživača te pokazati vlastita iskustva iz tog područja.

Laparoskopija je postala važan alat za proučavanje fiziologije reprodukcije još od 1968. kada je Roberts izvijestio o prvom endoskopskom pregledu spolnog sustava ovaca. U početku se, kao tehnika za izravno promatranje jajnika i maternice, koristila za proučavanje ovulacije, razvoja folikula i žutih tijela te za dijagnostiku gravidnosti. Paralelno s brzim razvojem metoda asistirane reprodukcije, laparoskopija je uvedena kao tehnika za poboljšavanje njenih rezultata. Salamon i Maxwell (2000) navode kako je od više metoda korištenih za poboljšanje plodnosti nakon osjenjenjivanja, najučinkovitije laparoskopsko intrauterino osjenjenjivanje. Ovom se tehnikom prevladao problem „cervikalne barijere“ te su dobiveni zadovoljavajuće stope plodnosti sa signifikantnom redukcijom broja spermija potrebnih za osjenjenjivanje (od 200-300 na 1-10 milijuna ili manje u slučaju seksiranog sjemena). Stopa gravidnosti u koza i ovaca uobičajeno premašuje 75 odnosno 50%.

Laparoskopiju je u MOET postupke uveo Mc-Kelvey i sur. (1986) s primarnim ciljem smanjenja adhezija reproaktivnog sustava nakon kirurškog dobivanja zametaka. Laparoskopija omogućuje ponavljana ispiranja ovčjih i kožnjih maternica uz zadovoljavajuću stopu uspješnosti ispiranja. Nadalje, dosegнуте su visoke stope gravidnosti nakon laparoskopskih transfera svježih ili otopljenih zametaka u metnicu ili jajovode primateljica. Preporučena je od strane mnogih autora kao minimalno invazivna kirurška tehnika. Laparoskopija je također korištena za aspiraciju folikula i dobivanje oocita (Laparoskopski ovum pick up – LOPU) u malih preživača.

Laparoskopija se u postupcima asistirane reprodukcije u Makedoniji počela koristiti u ranim 90 godinama 20. stoljeća nakon nabavke prve laparoskopske opreme. Nakon početnog razdoblja usvajanja rutine laparoskopija je uključena u više znanstvenih projekata za potrebe i ntrauterinog osjenjenjivanja, ispiranje maternica te transfer zametaka u ovaca i koza. Kasnije je pokušano uvesti laparoskopsko osjenjenjivanje u rutinsku farmsku praksu. Intrauterino / intrakornualno osjenjenjivanje ovaca otopljenim sjemenom dovelo je do stopa gravidnosti od 45 tj. 60% pri osjenjenjivanju van sezone, odnosno tijekom sezone spolne aktivnosti. U koza su ti postotci nekad i viši (preko 80%).

Zaključno, laparoskopija može biti kosistrišni dodatni alat u postupcima asistirane reprodukcije malih preživača, iako ova tehnika ima ograničenja u rutinskoj praksi jer zahtijeva ekspertna znanja i povišene troškove.