TRANSVAGINAL ULTRASOUND GUIDED PUNCTURE OF FOLLICLES AND OOCYTES ASPIRATION IN HEIFERS BY A MODIFIED DEVICE

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Recovery of oocytes from ovaries of slaughtered animals represents a non-repeatable source of ova for in vitro fertilization and obtained embryos are mostly of less genetic interest for breeding programs (Pieterse et al., 1991, Kruip et al., 1994, Bols et al., 1995, Gibbons et al., 1995). Laparoscopic follicular oocyte aspiration combined with ultrasonography has also limitations due to scar tissue formation and adhesions at the operation site and in the ovaries (Pieterse et al., 1991, Gibbons et al., 1994, Looney et al., 1994, Bungartz et al., 1995, Stubbings & Walton, 1995).

The ultrasound guided transvaginal follicular aspiration has been developed as an alternative method for obtaining oocytes from live animals (Pieterse et al., 1988). The method allows collection of oocytes with high degree of repeatability from animals of known genetic merit at any stage of the estrous cycle. This can be done with or without stimulation of follicular growth and from follicles at different stage of development over a period of several months (Pieterse et al., 1988, Pieterse et al., 1991, Walton et al., 1993, Bergfelt et al., 1994, Kruip et al., 1994, Looney et al., 1994, Vos et al., 1994, Pavasuthipaisit et al., 1995, Paul et al., 1995, Brogliatti et al., 1995, Bungartz et al., 1995, Dolman et al., 1995, Gibbons et al., 1995). The technique is more rapid and less traumatic for animals than aspiration of oocytes by laparoscopy (Pieterse et al., 1988, Pieterse et al., 1991, Bols et al., 1995, Stubbings & Walton, 1995).

The aims of this study were to evaluate the function and the practicality of a technically modified device for ultrasound guided puncture of follicles and oocyte aspiration and to determine possible traumatic effects on the genital tract during long-term repeated oocyte aspirations in heifers.

Material and Methods

Two heifers of the Swedish Red and White breed (13 and 14 months of age) were used in the experiment. The heifers were housed indoors in the barn of the Department of Obstetrics and Gynaecology (Swedish University of Agricultural Sciences) and fed with good quality hay and concentrate, plus mineral supplement, according to Swedish standards.

An ultrasound sector scanner (Scanner 250, Pie Medical, The Netherlands), with an endovaginal multiple angle transducer (Pie Medical, The Netherlands, 5.0-7.5 MHz) was used. The transducer was inserted into a specially made stainless steel holder (length 60 cm, inner diameter 32.1 mm and outer diameter 38 mm) equipped with a ventrally positioned single lumen needle guide.

The needle system for puncture of follicles consisted of two parts, a 58 cm long single stainless steel tube (inner diameter 1.2 mm and outer diameter 1.8 mm) and a 5 cm long disposable needle (inner diameter 0.8 mm and outer diameter 1.1 mm). The puncture needle was attached in the end of the needle guide.

The transducer and needle guidance holder were equipped with a handgrip (13.5 × 3.0 × 4.5 cm) which was designed to allow the movement of the puncture needle forth and back by a manually activated trigger, inserted in the handgrip. Before use, a guidline for the puncture needle was marked on the screen by testing the unit in water. The handgrip allowed the operator to manipulate and to fix the unit in the required position inside the vagina and, without assistance, to insert the needle through the vaginal wall into the visualized follicles.

For aspiration of follicular fluid and oocytes the distal end of the steel tube was connected by silastic tubing (inner diameter 1.0 mm and outer diameter 2.0 mm, Corning Corp, USA) to a heparinized blood collection test tube (Venoject, Terumo Europe N.V., Belgium) with a small volume of phosphate buffered saline (PBS). The tube was through another silastic tubing connected to an electric suction pump (De Vilbiss Co, USA). Before being used, the steel tube and silastic tubing were rinsed with PBS plus heparin to prevent coagulation of the follicular fluid.

The heifers were restrained in a crate in a standing position and sedated intravenously with Domosedan 1 mg/100 kg bodyweight (Orion Corp., Finland) to minimize their movement during aspiration. Epidural

anesthesia was performed by administration of 4 ml Xylocain adrenalin (Astra Läkemedel, Sweden) to prevent rectal contractions. After evacuaton of faeces from the rectum and thorough cleaning of the vulva and perineum, the aspiration device, covered with a latex cover containing contact gel, was inserted into the vagina and placed on the left or right side of the external os of the cervix.

The ovary subjected to aspiration was positioned by manipulation per rectum on to the head of the transducer so that the follicle was transected by the biopsy line on the monitor. The needle was thereafter pushed through vaginal wall into the follicle. Continuous suction was started as soon as the needle tip was seen in the follicle. After the follicle had collapsed, the needle was withdrawn and the next follicle was subjected to aspiration.

At each collection session follicles larger than 3 mm in diameter were punctured. When aspirations were finished, the needle was removed from the holder and flushed with PBS plus heparin into the same collecting tube. Oocytes were recovered from the follicular fluid under a stereo microscope (Wild, M-8, Switzerland).

A week after the last aspiration the heifers were slaughtered and their genital tracts and ovaries subjected to gross examination. The ovaries were fixed in 10 % formaline, and routinely embedded in paraffin. Paraffin wax sections (5 μm) were stained with haematoxylin and eosin to aid microscopic evaluation of possible ovarian lesions after puncture.

Results and Discussion

In the present study attention was paid to technical aspects of oocyte aspirations with a newly modified device for ultrasound guided follicular puncture. The major difference of the present device compared to other instruments which have been presented (Pieterse et al., 1988, Gibbons et al., 1994, Looney et al., 1994, Bols et al., 1995) is the possibility for the operator to control the movement of the aspiration needle. In most other instruments presented, the puncture of the follicles is performed by an assistant who manually pushes the needle into the follicles (Pieterse et al., 1988, Gibbons et al., 1994, Scott et al., 1994, Bungartz et al., 1995).

It is advantageous if the same person controls both the direction of the probe and the puncture of the follicles. A second advantage with the present unit is that disposable needles are used which are simply changed after each puncture session.

In the experiment, follicles larger 3 mm in diameter were punctured only in two heifers. This resulted in a limited total number of follicles punctured and oocytes aspirated. Also the person performing the puncture of follicles had no experience of the technique before the start of the experiment.

During the first 6 weeks the recovery rate of oocytes (percentage of oocytes recovered out of follicles punctured) was 36.4 % (4/11). Following next 6 weeks a recovery rate of 41.4 % (8/18) was achieved and after the last 4 weeks the recovery rate reached 73.7 % (14/19). In total the aspiration of oocytes during 16 weeks resulted in a recovery rate of 52.2 % (26/48). Our results show an increasing recovery rate by around 100 % during 16 weeks ending up with final recovery rate of 74 % which is comparable with other reports (Looney et al., 1994, Bungartz et al., 1995).

Gross examination of the genital tracts following slaughter one week after last aspiration revealed a haematoma on the cranial side of vagina in one heifer and, in the other, minor fibrous adhesions at the site of puncture of the vaginal wall. Histological examination of ovaries revealed an increased amount of connective tissue in one of the four ovaries.

Revealed minor traumatic changes are in agreement with other reports (Gibbons et al., 1994). It is unlikely that the morphological changes found in the two heifers in the present experiment would have influenced their future fertility. This assumption is confirmed by a study made by Metthews et al. (1995) where all 31 heifers subjected to repeated ovum pick up were inseminated after the end of the sessions and all heifers became pregnant within 68 days after the last ovum pick up session.

Conclusions

In conclusion, our results show that the hereby presented device for ultrasound guided ovum pick up enables a single operator to control both the ultrasound transducer and puncture needle without causing any major trauma to the genital tract. A person with no experience of ovum pick up can reach acceptable recovery rate of oocytes after about three months training.

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Transvaginaalne folliikulite punkteerimine ja ovotsüütide aspireerimine ultraheli kontrolli all mullikatel

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Kokkuvõte

Töö eesmärk oli uurida meie poolt modifitseeritud instrumendi sobivust ultraheli kontrolli all toimuvaks transtservikaalseks antraalsete folliikulite punkteerimiseks ja ovotsüütide aspireerimiseks elusloomadel ning selle protseduuri võimalikku traumaatilist mõju suguelunditele. Võib väita, et meie poolt modifitseeritud instrumendi eeliseks võrreldes senikasutatuga on see, et ta võimaldab operaatoril üksi, ilma abilisteta, teostada transvaginaalset folliikulite punkteerimist ja ovotsüütide aspireerimist elusloomadel. Selle instrumendi kasutamisel oli võimalik punkteerida kõiki folliikuleid, mille diameeter oli 3 mm või enam ja ovotsüüte saadi enamikust (73,7 %) punkteeritud folliikulitest. Morfoloogiliste ja histoloogiliste uuringute põhjal võib märkida, et folliikulite korduva punkteerimise järel tekkinud muutused tupeseinas ja munasarjas ei ole märkimisväärsed.