Transvaginal interstitial laser treatment of the ovary: a feasibility study in cows

Eugenie M. Kaaijk¹,²,⁵, Maarten C. Pieterse⁴, Johan F. Beek¹, Fulco van der Veen⁴, Fibo J.W. ten Kate³, Frits B. Lammes² and Martin J.C. van Gemert¹

¹Laser Centre, ²Divisions of Gynaecology and Reproductive Endocrinology, Meibergdreef 9, 1105 AZ Amsterdam, ³Pathology, Academic Medical Centre, Amsterdam and ⁴Department of Herd Health and Reproduction, Faculty of Veterinary Medicine, University of Utrecht, Utrecht, The Netherlands

In 12 cows, transvaginal interstitial laser treatment (TILT) of the ovaries was performed using a neodymium:yttrium aluminium-garnet laser to investigate the feasibility of a new treatment approach for clomiphene-resistant patients with chronic hyperandrogenic anovulation. Powers of 1 and 2 W during 5 min of exposure were used. Sonographic changes of thermal damage during TILT, the extent and healing of the lesions by light microscopy and ultrasound during a 3 month follow-up and adhesion formation were studied. During laser irradiation, a hyperechogenic zone developed around the fibre tip, with a mean ± SD diameter of 4.4 ± 2.0 mm at 1 W and 6.9 ± 1.5 mm at 2 W. The mean diameters of the histological lesions 2 days after treatment were 7.3 ± 2.5 mm at 1 W and 13.0 ± 2.1 mm at 2 W. During follow-up, the mean diameter of both the histologically and the sonographically assessed lesions decreased, although transvaginal sonography (TVS) systematically and significantly underestimated the thermal damage. Lesions healed by fibrosis and no adhesions were present. TILT of the ovaries in cows is easy to perform and produces central or subcapsular necrosis without adhesions. TVS gives an indication of thermal damage but underestimates the extent of tissue damage in cow ovaries. Obviously, this study does not allow conclusions to be drawn concerning its safety and efficacy in man.

Key words: adhesion formation/chronic hyperandrogenic anovulation/Nd:YAG laser/transvaginal sonography

Introduction

Ovulation in clomiphene-resistant patients with chronic hyperandrogenic anovulation (CHA) can be restored by laparoscopic electrosurgery or laparoscopic laser surgery of the ovary. In a review of the literature, ovulation was induced in 70–78% of patients after various laparoscopic treatments, and pregnancy was achieved in ~40% of patients (Kaaijk et al., 1995). However, despite the promising results, these treatments have several drawbacks. Firstly, they cause extensive areas of injury to the ovarian surface, thereby increasing the risk of adhesion formation (Dabirashrafi et al., 1991; Gurgan et al., 1991, 1992; Greenblatt and Casper, 1993; Naether and Fischer, 1993). Secondly, the restoration of ovulation in patients who do not conceive is of limited duration (Gjönnæs, 1984). Thirdly, a laparoscopy with general anaesthesia is needed for these procedures. Transvaginal interstitial laser treatment (TILT) of the ovary, a new minimally invasive treatment modality for ovulation induction in these patients, may by-pass the drawbacks of the laparoscopic techniques.

Until now, interstitial laser treatment (ILT) has been used clinically to destroy tumours and metastases in the brain (Betttag et al., 1991; Fan et al., 1992), the liver (Hashimoto et al., 1985; Huang et al., 1991; Masters et al., 1992; Amin et al., 1993), the pancreas (Steger et al., 1989), the skin (Steger et al., 1989), head and neck regions (Castro et al., 1992; Blackwell et al., 1993) and the breast (Harries et al., 1994) by placing a laser fibre [coupled to a neodymium:yttrium aluminium—garnet (Nd:YAG) laser] into the tumour tissue. Several studies reporting on the use of ultrasound to visualize thermal damage during and after ILT found a correlation between the extent of hyperechogenic zones and the extent of thermal necrosis measured histologically in healthy tissue (Goedewysky et al., 1988a,b; Bosman et al., 1991; Steger et al., 1992). TILT of the ovary involves a transvaginal puncture of the ovaries under ultrasound guidance and positioning of a laser fibre through the puncture needle into the ovary. Hypothetically, this treatment will result in a reduction of ovarian androgen production caused by the coagulation of ovarian theca cells with only minimal capsular damage, and therefore a minimal risk of adhesion formation. Transvaginal sonography (TVS) might be used to monitor the extent of thermal damage.

In a pilot study concerning laparoscopic ILT of the ovary in goats, it was shown to be feasible to create intra-ovarian lesions with only minimal capsular damage and no adhesion formation at powers of 1 and 2 W during 5 min. However, a transvaginal approach including ultrasound monitoring of the thermal damage was not possible in this animal model (Beek et al., 1995).

The aims of this study were (i) to investigate the feasibility of monitoring thermal damage during TILT by TVS, (ii) to compare the lesion diameters measured histologically and sonographically during a 3 month follow-up study and (iii) to investigate the healing of the lesions and adhesion formation.

Materials and methods

Laser and ultrasound equipment

A continuous wave Nd:YAG laser (Medilas 40N; MBB, München, Germany) with a wavelength of 1064 nm was used. The laser light...
was delivered into the ovary by a bare 600 μm diameter quartz fibre. An ultrasound sector scanner (SDR 1550; Philips, Eindhoven, The Netherlands) was used with a 7.5 MHz vaginal transducer, which was extended to a length of 50 cm and equipped with a needle guide. A sterilized needle with a sharp tip was used to puncture the ovary through the vaginal wall and to guide the laser fibre (external diameter 1.2 mm, internal diameter 0.8 mm).

Animal techniques
A group of 12 cows was selected by an assessment of the size and mobility of their ovaries by rectal palpation. The animals were housed together in open stables of the Department of Herd Health and Reproduction at the Faculty of Veterinary Medicine, Utrecht, The Netherlands. All cows were synchronized hormonally by receiving an ear implant (Crestar®) containing progesterone (Norgestomet, 3 mg) 10 days before the start of the procedure. The ear implant was removed on the day of treatment. Just before treatment the cows were premedicated with an i.v. injection of 0.1 ml/100 kg Domosedan® (detomidine hydrochloride 10 mg/ml; Smith Cline Animal Health Ltd, Intervet Netherland BV, The Netherlands) for sedation and relaxation of the rectum. An epidural mixture of 5 ml lidocaine (2%) and adrenalin (2%) was administered to prevent abdominal straining. Before the procedure the rectum was emptied and the vulva and perineum were cleaned thoroughly to prevent any contamination (Pieterse et al., 1988). During the procedure the cows were standing and were restrained to prevent them from moving.

Laser treatment
Both ovaries of all cows were irradiated during 5 min, the larger at 2 W and the smaller at 1 W. These powers were based on a previous study in goats (Beek et al., 1995). The ultrasound probe was inserted into the vagina and one ovary was positioned manually against the head of the probe by rectal manipulation. The needle was inserted through the needle guide into the ovary, followed by approximate placement of the laser fibre in the centre of the ovary. After placement of the fibre the needle was withdrawn gently for ~1 cm to prevent heat conduction along the needle to the surface of the ovary during laser irradiation. After each treatment the fibre tip was inspected for carbonization or damage. During all laser irradiations the ovaries remained positioned against the ultrasound probe. Sonographic images obtained during TILT were visualized on a monitor screen and recorded on a videotape. Furthermore, photographs of the sonographic images were taken before, during and immediately after laser treatment. The long and short axes of the sonographic lesions were measured by a radiologist experienced in ILT in the liver, who was blinded for the powers used and the duration of TILT. For each lesion, the mean of the long and the short axes (the diameter) was considered. In one series (e.g. all ovaries treated at 1 W) the mean of these diameters (±SD) was considered.

Follow-up study
Sonographic evaluations of the lesions in the ovaries were performed on days 2, 7, 14, 28, 52 and 81 after treatment. Furthermore, follicular growth was monitored during the sonographic evaluation. The long and short axes of the sonographic lesions were measured from photographs by the radiologist who was blinded for the powers used and the day of follow-up. Again, the diameter (mean of the long and short axes) and the mean diameter for one series were considered. After each evaluation, two cows were slaughtered and the ovaries removed. Therefore 2 days after laser irradiation 12 cows were evaluated sonographically and two cows were slaughtered, 7 days after irradiation 10 cows were evaluated sonographically and two cows were slaughtered, and so on. After an inspection for capsular damage and adhesion formation, the ovaries were stored in buffered formalin (10%, pH 7.4). After fixation, the ovaries were serially sectioned tangential on the ovarian hilus in slices with a thickness of 5 mm, embedded in paraffin, cut in sections (with a thickness of 5 μm) and stained with haematoxylin and eosin for light microscopic examinations. In a similar way to that described for the sonographic lesions, the histological lesions were evaluated by one pathologist. The correlation between the sonographic lesions and the histological lesions was determined by a paired Student’s t-test.

Results
TVS before laser irradiation showed ovaries with only a few small follicles because of the Crestar® ear implant. The transvaginal puncture technique was easy to perform. A total of 23 out of 24 ovaries were treated. One ovary was too small (1.0×1.0×1.5 mm) for puncturing. During all laser irradiations a hyperechogenic zone developed around the fibre tip, which increased in size during exposure (Figure 1A-C). The onset of the sonographic changes varied: in six out of 23 ovaries the hyperechogenic zone developed within a few seconds of irradiation, while in the remaining ovaries hyperechogenic zones developed at between 15 and 260 s of irradiation. A time series of the mean extent of hyperechogenic zones obtained at 1 min intervals during TILT is shown on the left-hand side of Figure 2. In some ovaries small echogenic spots, probably corresponding to vapour bubbles, were seen moving away from the lesion during laser irradiation. In some cases, a relatively minimal temperature increase on the surface of the ovary was felt manually during the transrectal fixation of the ovary at treatment. TVS on day 2 showed clearly visible, circumsrptive hyperechogenic zones which were easy to characterize. The right-hand side of Figure 2 shows the mean diameters of the lesions assessed sonographically during follow-up. An increase in the mean diameter of the hyperechogenic zones was noticed 2 days after the procedure in five out of 12 ovaries irradiated at 1 W and in five out of 12 ovaries irradiated at 2 W. After day 2, the mean diameter of the hyperechogenic zones as well as the echogenicity of the zones decreased at both powers. Hyperechogenic zones were seen sonographically until day 14 at 1 W and until day 28 at 2 W. Macroscopic evaluations of the ovaries removed on days 2 and 7 after laser treatment showed a small lesion on the ovarian surface which corresponded to the puncture site of the needle. Ovaries removed after 7 days did not show any capsular damage. None of the ovaries showed adhesions during follow-up. Serial sections of the ovaries showed central or subcapsular lesions which were easily visible in the first 2 weeks. Light microscopic evaluations on day 2 showed sharply demarcated lesions. Lesions performed at 2 W consisted of a small central cavity lined with carbonized debris. Around this cavity there was a zone of coagulative necrosis with condensed, hyperchromatic nuclei and a zone characterized by dilated blood vessels. Lesions produced at 1 W showed neither cavity formation nor carbonization. On day 7, the haemorrhagic necrotic area was infiltrated by an inflammatory infiltrate (Figure 3B). On days 14 and 28, fibrin deposits were noticed within the coagulation zone which was surrounded by a zone of fibrosis with proliferating blood vessels. In the centre of the lesions, giant...
cells were seen around the carbonized tissue. On day 52, fibroblastic ingrowth continued; on day 81, lesions were healed by fibrous nodules of $2.0 \pm 0.1$ (mean $\pm$ SD) and $5.0 \pm 1.4$ mm for 1 and 2 W respectively (Figure 4). These lesions could no longer be detected by the naked eye at this stage. All ovaries resumed normal follicular growth during follow-up studies.

Figure 5A and B compares the mean diameters of the lesions, measured both histologically and sonographically, of 12 cows at 1 W (Figure 5A, one data point represents two cows) and of 11 cows at 2 W (Figure 5B, data point at 52 days concerns only one cow) just before killing the cows. Histologically, lesions produced at 2 W were significantly larger than those produced at 1 W ($P < 0.05$). The hyperechogenic zones produced at 2 W were larger than the hyperechogenic zones produced at 1 W during the first week of follow-up. The extent of hyperechogenic zones compared with the extent of histologically measured lesions was consistently and significantly smaller at both powers ($P < 0.01$).

Discussion
The main purposes of this study were to investigate the feasibility of monitoring thermal damage during TILT of the ovary by TVS in an animal model, and to compare quantitatively the sonographic changes with the extent of macroscopic and light microscopical changes. The cow was chosen for two reasons. Firstly, the size of a cow ovary corresponds to the size of a polycystic ovary in patients with CHA. Secondly, transvaginal puncturing of the ovary under ultrasound guidance is an established technique for in-vitro fertilization purposes in cow reproduction (Pieterse et al., 1988).
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Figure 2. The mean diameter of the hyperechogenic zones as a function of time during laser irradiation and during follow-up. For each lesion, the average of the short and long axes was considered. For each time period, the mean of these averages is given ±SD, including the evaluation of 12 cows at 2 days, 10 cows at 7 days, and so on.

Figure 3. Macroscopic (A) and light microscopic overview (B) of an ovary 7 days after laser irradiation at 1 W. The thermal lesion is sharply demarcated from normal ovarian tissue. There is coagulative necrosis surrounded by a zone of haemorrhage. Original magnification ×2.

The sonographic changes observed during treatment were similar to the results of several other animal experimental studies concerning the monitoring of thermal damage by ultrasound during ILT in normal liver (Godlewsky et al., 1988b; Bosman et al., 1991; Malone et al., 1992; Steger et al., 1992). However, in those studies the extent of the sonographic lesions corresponded well with the extent of the lesions measured histologically, while in our study the extent of thermal damage was systematically and significantly underestimated by TVS. Because it was impossible to slice the ovaries along the fibre-optic tract after resection (the puncture site of the needle was no longer visible after 7 days), the differences in the extent of sonographically and histologically assessed lesions may be partly caused by the different planes of measurement. However, another explanation for not finding a good correspondence might be the heterogeneity of the ovarian tissue. In a recent clinical pilot study concerning ultrasound-guided ILT of small mammary carcinomas, Harries et al. (1994) found no relationship between the lesions assessed by sonography and those assessed by histology. The main reason for this phenomenon was assumed to be the heterogeneity of the tumour tissue (S.G.Bown, personal communication).

The mechanism which causes the sonographic changes during laser irradiation is not well established but may be due to the heating of tissue and the denaturation of proteins. Malone et al. (1992) implanted microthermocouples at 4 and 8 mm from the fibre tip and measured temperatures during ultrasound-guided ILT in a pig liver. The heated tissue became noticeably hyperechogenic if temperatures exceeded a threshold of 50°C. During irradiation, the threshold temperature was attained at increasing distances from the fibre tip, resulting in an increasing hyperechogenic zone.

Two authors have described the phenomenon of target lesions, i.e., lesions consisting of different layers of echogenicity on ultrasound within 10 days of ILT (Godlewsky et al., 1988b; Bosman et al., 1991). The different layers of echogenicity corresponded to different histopathological layers and consisted of cavitation, necrosis and fibrosis respectively. In our study, target lesions were only found in two ovaries 2 days after laser irradiation. However, these ovaries were not investigated histopathologically at that time.

The cavity formation and charring of the tissue on histological examination of the ovaries after irradiation at 2 W suggest that high temperatures have been reached around the fibre tip during irradiation. Despite these possibly high temperatures, only a minimal temperature increase on the surface of the ovary was felt manually during transrectal fixation and irradiation. However, these findings cannot be used to draw conclusions concerning the safety of this procedure in humans. If temperatures on the capsule of the ovary become too high, there is a serious risk of capsular damage, thermal necrosis of the intestines and destruction of the ovarian hilus, resulting in ovarian atrophy and loss of ovarian function.
Figure 5. The mean diameters (±SD) of the histological lesions of 12 cows at 1 W (A, one data point represents two cows) and of 11 cows at 2 W (B, data point at 52 days concerns only one cow) and of the mean diameters of the corresponding sonographic lesions of the same two cows (except for the data point at 2 W and 52 days) just before killing the cows. The histological lesion is significantly underestimated by transvaginal sonography (paired Student's t-test, \( P < 0.01 \)).

In agreement with other studies concerning the healing process of laser-induced lesions, we observed sharply demarcated necrotic lesions on histological examination on day 2, the initial growth of the lesions (probably caused by oedema), the decrease in size of the lesions and the healing by fibrosis during follow-up (Matthewson et al., 1987; Godlewsky et al., 1988b; Bosman et al., 1991). The resumption of follicular growth and ovulation after removal of the progesterone-containing ear implant shows that lesions in the ovaries of cows heal safely after TILT. The minimal capsular damage and the absence of peri-ovarian adhesion formation after TILT strengthen our hypothesis that ILT with the avoidance of capsular damage causes a minimal risk of adhesion formation. Because post-operative adhesion formation is still a matter of concern using the current surgical techniques for ovulation induction in patients with CHA, in which multiple superficial lesions are created through the ovarian capsule, this result is potentially of great value. However, significant conclusions concerning the absence of adhesion formation cannot be made because of the low number of only two cows per time interval.

In summary, TILT of the ovary in cows is feasible. It is easy to perform and produces central or subcapsular necrotic lesions with only minimal capsular damage. Lesions healed safely and no adhesion formation occurred after TILT of the ovary in our study. TVS gives an indication of the thermal damage during TILT, but it systematically and significantly underestimates the extent of tissue damage in cow ovaries. We suggest that TILT may in the future offer a new, minimally invasive treatment technique for clomiphene therapy-resistant patients with CHA. However, it should be emphasized that our study does not address its safety and effectiveness in the human. Therefore, clinical evaluations should be carried out very carefully in a well-designed manner under laparoscopic control before the safety of the technique can be assessed.

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