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Evaluation of three polymer materials for the production of embryo transfer catheter for pigs

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Summary

The research aimed to improve laparoscopic methods of embryo transfer in pigs and implement new biomaterials with high biocompatibility and desired material characteristics for reproduction. The possibility of using biometrics used in the medicine of polypropylene (PP), polyethylene (PE), and polylactide (PLA) was investigated. In the first stage, material tests were carried out, determining the functional properties of the materials and the possibility of forming tubes with the desired mechanical properties (strength and flexibility), geometry (length, thickness, and shape constancy), and surface structure. In the second stage, the functional features and biocompatibility of the materials were confirmed by the recipients. Of the materials tested, only the PE catheters had functional and biological properties. An efficient method of laparoscopic embryo transfer in pigs has been developed using the developed PE catheter.

Keywords: embryo, transfer, bimaterials, catheter, pig

In recent years, there has been significant progress in the field of assisted reproductive technology (ART) (30). They include procedures related to the collection, breeding, embryo freezing, artificial insemination, *in vitro* fertilization and embryo transfer (1, 17). The development of these areas of reproduction is not possible without simple and effective methods of embryo transfer with the possibility of wide practical application. Surgical methods of embryo transfer in pigs used so far are of limited use due to significant tissue trauma, postoperative complications, long healing time, high cost and long implementation time, the need for qualified personnel and the risks associated with general anesthesia (13, 18, 21, 28).

An alternative to these procedures are minimally invasive, endoscopic, orlaparoscopic methods. Compared to surgical methods, these techniques allow, above all, to significantly reduce the invasiveness of the procedure and eliminate the possibility of secondary post operative complications, therefore, they are safe for animals and additionally allow for shortening the procedure time (16), provide easy access to the middle or end sections of the uterine horns, enable multiple use of the procedure in the same animals and significantly reduce the risk of transmitting infectious diseases (19, 21). In the case of laparoscopic embryo transfer in pigs, the main obstacle is the specific anatomy of the fallopian tubes and the uterus. The exceptional length of the uterine horns up to 100 cm, as well as the long and tortuous fallopian tube, means that laparoscopic methods in pigs require specialized tools, are technically extremely difficult and require a lot of manual skill from the operators. The methods described in various variants have been described in

the world for about 30 years (7, 24, 25, 31, 33). The effectiveness of these methods varies from a dozen to about 50% of pregnancies (16, 27). Various types of catheters are used for embryo transplantation, e.g., human or often adapted and modified artificial insemination catheters, Venflon catheters or Folley catheters (7, 14, 16, 27, 31, 35). The significant diversity of catheter models and the very different transfer efficiency indicate the need to improve embryo transfer methods in animals and to search for new types of catheters. An ideal ET catheter should be biosecure, non-embryo toxic, soft enough to avoid trauma to the lining of the fallopian tube and uterus, but also flexible enough to be inserted to the desired length in the lumen of the uterus or fallopian tube along their natural path and provide the possibility of embryo transfer in a very short time, up to 2-4 minutes, in a minimum amount of about 20 µl of medium (2, 4, 6). Such a possibility is provided by the introduction of catheters made of biomaterials with different physico chemical and mechanical properties than those used so far. The use of bio materials in veterinary medicine is common and the characteristics of the materials are known; their bio compatibility with respect to living tissues has also been confirmed (22). However, its use in reproduction is very limited and there has been no research focusing on the use of new biomaterials in this veterinary field. The purpose of the research carried out was to find new solutions and materials for the production of embryo transfer catheters. Due to the high sensitivity of the embryos, the fallopian tube, and the uterus, the requirements for such biomaterials are, first of all, the lack of cytotoxicity, but also the defined physicochemical properties of the surface, the possibility of free formation, adequate strength and flexibility, and ease of use. The material tests carried out allowed for a comprehensive determination of the usefulness of the proposed solutions in terms of determining the physical properties of selected biomaterials and the possibility of producing appropriate catheters from them. However, the comparative biological research carried out determined the possibility of practical application of catheters for the transfer of embryos made of selected biomaterials. The results of the research allowed for the production of a catheter with the desired biological and physical characteristics and the effective implementation of embryo transfer.

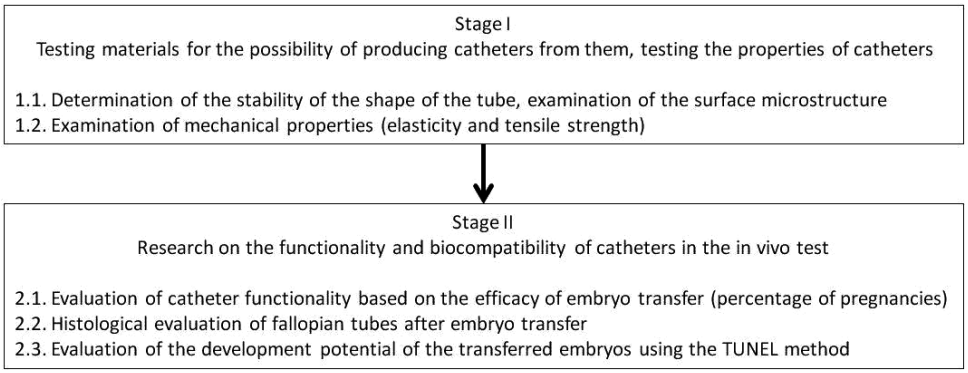

Fig. 1. Experimental protocol, in two parts: I – testing the mechanical properties of catheter proteases and II – testing the functionality of catheter proteases on animals

Material and methods

The layout of the experience. All procedures were performed with the prior approval of the II Animal Ethics Committee in Krakow, approval numbers 791/2010. Research was carried out in two stages (Fig. 1). In the first stage, the possibility of obtaining a catheter with the desired physical properties was determined: appropriate length, shape stability, surface smoothness, tensile strength, and flexibility. The study used 3 types of commercial polymers: low density polyethylene (PE) (ULPE, Mw = 85 kDa, BASF), high molecular weight polypropylene (PP) (PP, Mw = 360 kDa, BASF) and a bioresorbable polymer: copolymer L/DL lactide (PLA, Mw 230 kDa, PURAC). In the process of producing catheters, a patent "A method of producing a catheter" was used (Patent 225658. Patent Office of The Republic of Poland 31.05.2017 r.; https://www.uprp.pl/strona-glowna/Menu01,9,0,index,pl/). The aim of this stage was to produce prototype catheters with a length of 15-20 cm and an external diameter corresponding to the diameter of the fallopian tube. 15 catheters of each type (n = 15) were used in the investigation. Due to the lack of standards and standards for gynecological catheters, material tests were performed according to the standards applicable to medical devices, urological and intravenous catheters (ISO 10555-1, 2013; ISO 20696, 2018). In the second part, embryo transfer to animals was performed with prototype catheters. In an *in vivo* test on live animals, the biocompatibility of selected biomaterials and the functionality of catheters made of them were investigated.

I. Testing materials for the possibility of producing catheters from them and testing the properties of catheters

Determination of the stability of the tube shape and examination of the surface microstructure. To determine the consistency of the shape of the tube produced throughout its entire length, a geometric evaluation of the catheters was performed using the KYENCE stereoscopic microscope. The surfaces tructure was determined by scanning electron microscopy (SEM, Nova nanoSEM 200, FEI).

Examination of mechanical properties. The mechanical test determined: elasticity (Young's modulus E) and tensile strength Rm. Mechanical tests were carried out with the use of the ZWICK 1435 universal test machine. The following test parameters were used: measurement base (tube length) 20 mm, tensile speed 40 mm/min, according to the standard (ISO 10555-1, 2013; ISO 20696, 2018).

II. Research on the functionality and biocompatibility of catheters in the *in vivo* test

In the *in vivo* model, embryo transfer in pigs was performed laparoscopically with prototypes of the catheters

produced. Effectiveness was determined based on the percentage of pregnancies obtained, and, in addition, a histological examination of the fallopian tubes was performed after transplantation and the biocompatibility of the catheters was confirmed based on the developmental potential of the transferred embryos. Additionally, the number of donors used, as well as the number and quality of the embryos obtained and transferred, were recorded.

Transplantations were performed with two types of catheter made of PP with a diameter of 1.67 mm and PE with a diameter of 1.1 mm. The tests were carried out on 51 gilts, 6-12 months old, weighing 80-110 kg (32 embryo donors and 19 embryo recipients). Recipients and donors were routinely synchronized with the sexual cycle. Donors of additional super-ovulation according to the scheme described earlier (34). Of 19 recipients, 14 after transplantation were left for pregnancy diagnosis, the remaining 5, 5 days after transplantation, were sacrificed, the transferred embryos were washed out and the fallopian tubes were collected for histopathological examinations.

The animals were fasted 24 hours prior to surgery and free water was available.

Animal anesthesia. Animals were subjected to general infusion anesthesia. The animals were premedicated with azaperone 3-5 mg/kg i.m. (Stresnil, Jannsen). General anesthesia was induced with xylazine 1-2 mg/kg i.v. (Xylapan 2%, Vetoquinol, Poland) and ketamine 5-10 mg/kg i.v. (Ketamine 10%, Biowet Puławy, Poland). The preparations were dosed according to the effectiveness of the action. Embryos in the stage of development of 2-4 blastomeres were surgically rinsed out of the fallopian tubes as previously described (34). The embryos collected were transferred to fallopian tubes within 2-3 hours of collection.

Evaluation of catheter functionality based on the efficacy of embryo transfer (percentage of pregnancies obtained)

Endoscopic transfer of embryos. Three trocars were inserted into the abdominal cavity. The endoscope camera was placed on the left side between 2-3 pairs of nipples and two atraumatic holders for stabilization: the first in the navel, the second on the right between the fourth and fifth pairs of nipples. An abdominal catheter was introduced at the level of the stabilized fallopian tube by abdominal puncture and puncture of the fallopian tube. The set for transplantation consisted of two needles, one for abdominal puncture, then a second needle for fallopian tube puncture was inserted through the needle, after fallopian tube puncture, a catheter with embryos was inserted into its lumen up to a depth of 5 cm. The embryos were placed in the initial section of the catheter (about 1 cm) in the minimum amount of medium possible. The embryos were deposited unilaterally in the right or left fallopian tube by injection into the fallopian tube. After transplantation, catheters and grips were removed in reverse order, at the end the endoscope camera after visual inspection of the abdominal organs. Single absorbable sutures (PGA 1, DKO117PG, Yavo Polska) were placed on the skin. The peritoneum and abdominal muscles were not sewn. The pregnancy was diagnosed between 28 and 31 days after surgery by ultrasound. The percentage of pregnancies achieved was estimated.

Histological evaluation of fallopian tubes after embryo transfer. On the fifth day after embryo transfer, one fallopian tube after transplantation with a polypropylene catheter and four fallopian tubes after transplantation with a polyethylene catheter. Additionally, 3 fallopian tubes were collected, to which the embryos were not transferred. The fallopian tubes were inserted into 10% formalin and 2-3 mm transverse sections were prepared and embedded in paraffin blocks (automatic tissue processor, Shandon Excelsion ES). The blocks were cut into 2-3 μm sections and placed on slides. The slides were stained with hematoxylin-eosin in an automatic stainer (Shandon Veristan Gemini). The preparations were evaluated under a light microscope.

Evaluation of the development potential of the transferred embryos using the TUNEL method. The embryos were obtained by washing the uterus from the same recipients who underwent histopathological examination of the fallopian tubes. The tunnel test was performed according to the method described earlier (9, 10). In Situ Cell Death Detection Kit used; fluorescein (Roche, Mannheim, Germany). In stained blastocysts, the number of stained cells (nuclei) and the number of apoptotic nuclei were determined. Blastocysts were evaluated under an Eclipse E600 epifluorescence microscope (Nikon, Japan) using a filter wavelength of 358-461 and 520 nm. On the basis of the number of apoptotic nuclei to the number of total nuclei, the apoptotic index was calculated.

III. Statistical analyses. Statistical analyzes were performed using Statistica 13.3 software (TibcoStatistica, Krakow, Poland). The data was statistically analyzed using the one-way ANOVA test. The test was used for independent samples. The variables were treated as independent samples.

Results and discussion

Dedicated materials: polyproylene, polylactide, and polyethylene have been used in human and veterinary medicine for clinical and experimental purposes for many years (12, 20, 22, 23, 29). However, as has been shown, the introduction of new materials for reproduction requires the use of materials that meet stringent material and biological criteria and a wide verification of material and biological properties. First, material tests were performed. Material tests were carried out in three directions and involved: the possibility of forming catheters of the appropriate shape and diameter (shape constancy), obtaining the appropriate surface smoothness, and surgical flexibility (tensile strength and susceptibility to deformation, flexibility). These tests were designed to check the convenience and usability of the surgical instrument during surgery. Depending on the material used, tubes with a diameter of 0.85-4.08 mm were obtained (the wall diameter depended on the selected biomaterial: PP, PE, PLA). The best material was PE. With this material, it is possible to adjust the diameter of the catheter below 1.00 mm and any length. Catheters made of this material are characterized by constant shape and low surface roughness. The roughness of the outer surface of the polyethylene pipes was low. SEM

revealed no deep surface damage, perforation, or catheter discontinuity. Small defects in the outer layer were considered insignificant and will not affect the safe use of the catheter (Fig. 2.1. A, B). In the case of PP catheters, it is possible to adjust the diameter to 1.3 mm, and these catheters are characterized by a stable shape, although the variability of the shape of these catheters was greater than that of PE catheters ($p < 0.05$). On the outer surface of the PP catheter, surface roughness and numerous discontinuities were visible (Fig. 2.2. A, B). Microscopic observations of PE and PP tubes confirmed the stability of the geometry, the tubes: maintaining the dimensional stability and lumen of PE and PP catheters independent of the length of the catheter (Tab. 1). In the case of PLA catheters, there were difficulties with catheter formation and they were for tubes longer than 10 cm. In the case of PLA pipes, pipes with a sufficiently small diameter and length have not been produced. The variability of the shape of the tubes obtained and the mean deviations of the diameter and surface area (cross section) between catheters made of PP, PE, PLA ($p < 0.05$) were also significant. In the case of PLA catheters, significant roughness was demonstrated with deep surface damage (Fig. 2.3 A, B, C). Due to significant surface roughness and lack of shape stability, PLA catheters do not meet the requirements of embryo transplant catheters.

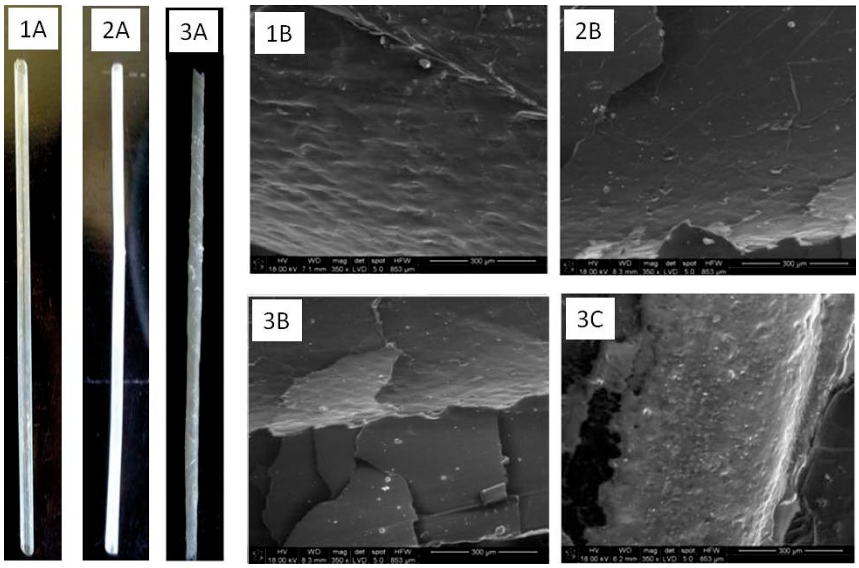

Fig. 2. Appearance of catheters and surface structure in SEM (Scanning Electron Microscope) examination. 1A – PE catheter, 2A – PP catheter, 3A – PLA catheter (rough surface structure visible), 1B – PE catheter surface in SEM test, 2B – PP catheter surface in SEM test, 3B and C rough PLA catheter surface with visible deep cavities

The insertion and removal of the catheter require adequate tensile strength and sufficient flexibility so that the catheter follows the shape of the fallopian tube. This feature ensures safe transport of embryos or gametes during the collection or implantation process and allows one to avoid mechanical damage to the fallopian tube (4, 6). The measure of mechanical properties was the strength of the catheter (tensile strength) and the Young's modulus, which defines the deformability (elasticity) (Fig. 3.). The change in

Tab. 1. Geometric characteristics and determination of the shape constancy of PE, PP and PLA catheters

Material	Diameter [mm] ± SD (min-max)	Δ diameter % ± SD (min-max)	Average of surface area [mm²] ± SD (min-max)	Δ surface area % ± SD (min-max)
PE	1.02 ± 0.1[a,b] 0.85-1.16	0.82 ± 0.06% –13-16%	0.82 ± 0.16[a,b] 0.57-1.06	6.63 ± 0.19% –27-29%
PP	1.74 ± 0.41[c] 1.28-2.3	6.49 ± 0.23% –32-28%	2.52 ± 1.15[c] 0.99-4.15	12.15 ± 0.44% –49-65%
PLA	3.15 ± 0.66 1.96-4.08	1.13 ± 0.2% –37.8-29.41%	8.12 ± 3.3 3.02-13.99	3.69 ± 0.4% –63-72%

Explanations: Δ – Change in diameter in surface area%; SD – standard deviation; a – statistically significant difference PE vs PP, $p < 0.05$; b – statistically significant difference PE vsPLA, $p < 0.05$; c – statistically significant difference PP vs PLA, $p < 0.05$

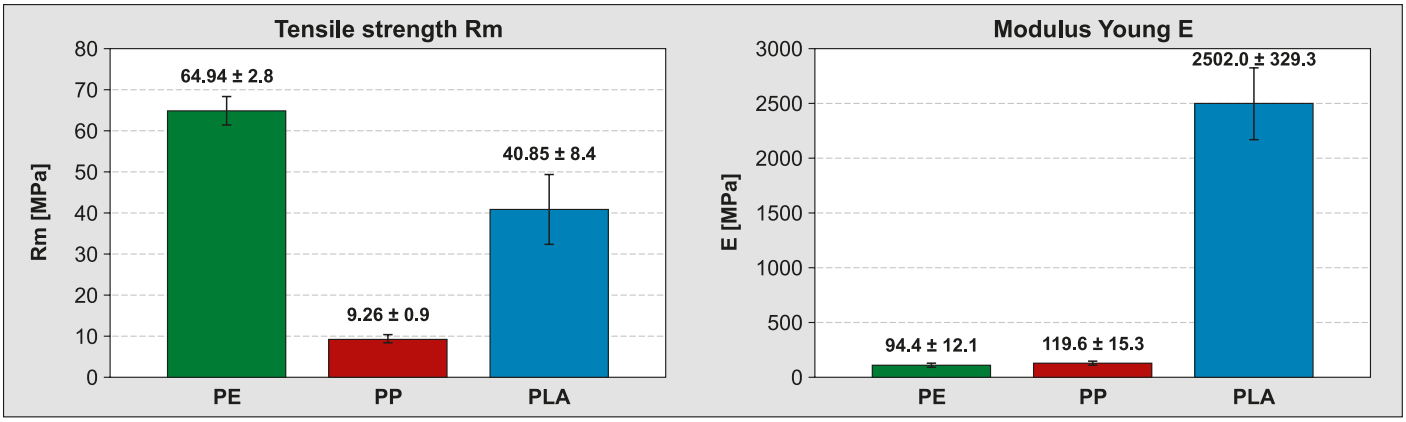

Fig. 3. Characteristics of the mechanical properties of PE, PP and PLA catheters

strength (tensile strength) varied in the series: PP < PLA < PE. Differences were statistically significant between all groups ($p < 0.05$). Polyethylene pipes are characterized by the highest tensile strength. Standard deviation valuesindicate that PE and PP catheters are highly homogeneous, while PLA catheters are not homogeneous in this respect. In the elasticity evaluation, it was shown that the PLA catheteris the least susceptible, followed by the PP catheter, and the PE catheter is the most flexible. The PLA catheter significantly exceeds the rigidity of the PP and PE catheter. Differences were statistically significant between all groups ($p < 0.05$). Taking into account the significant material defects of the PLA catheter, thiscatheter was eliminated at this stage and was not tested in an *in vivo* model.

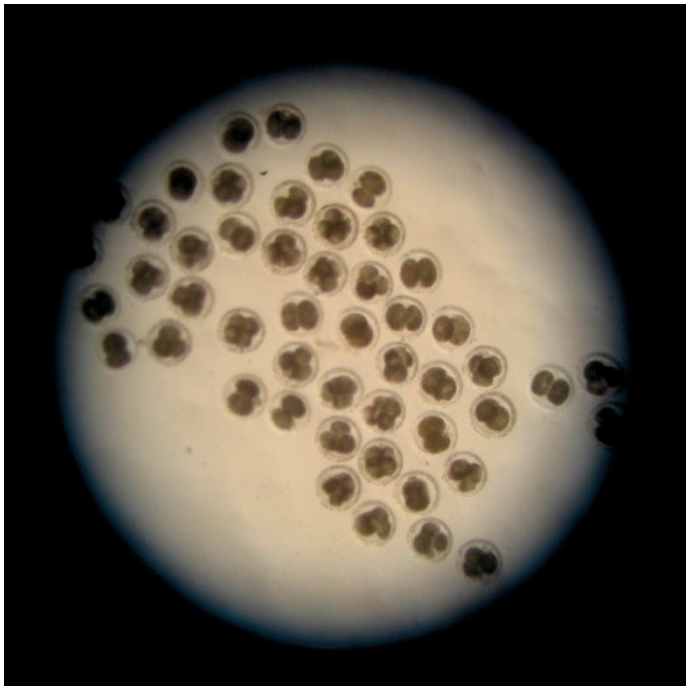

Fig. 4. Stage 2-4 blastomere embryos are surgically rinsed from the donor's fallopian tubes and transferred laparoscopically to the recipient's fallopian tubes (50 ×, Nikon SMZ-10A, Japan)

Tab. 2. Histological examination of fallopian tubes after embryo transfer with PP and PE catheters (histological changes were classified on a 4-point scale: 0 – none, 1 – small, 2 – moderate, 3 – considerable)

Histological change	PP	PE	Control
Preserved the lumen of the fallopian tube	3.0	3.0	3.0
Inflammatory infiltrates	3.0	0.25	0.3
Granulocyte infiltration in the fallopian tube	0.0	0.25	0.0
Granulocyte infiltration in the wall	3.0	0.5	0.0
Nonspecific granulation and mixed cell tissue in the wall	3.0	0.0	0.0
Epidermis in the lumen of the fallopian tube	3.0	0.0	0.0
Fatty tissue in the lumen of the fallopian tube	3.0	0.0	0.0
Purulent lesions of the fallopian tube	0.0	0.0	0.0
Purulent lesions around the fallopian tube	0.0	0.25	0.0
Granulocytes in the vessels	0.0	0.25	0.0

In the second part, in an animal study, 504 pig embryos were collected from 32 donors (Fig. 4). After evaluation, 475 embryos (94.2%) at the developmental stage of 2-4 blastomeres were transferred laparoscopically, the remaining 29 were disqualified due to lack of division or degenerative features.

Embryos were transferred to 19 recipients, 5 with a PP catheter and 14 with a PE catheter. Of 19 recipients, 14 (n = 4 PP and n = 10 PE) were diagnosed after 28-31 days, and the remaining 5 (n = 1 PP and n = 4 PE) underwent a histological examination of the fallopian tubes after 5 days. And the developmental potential of transferred embryos was studied.

On average, 28 embryos were transferred to one recipient. The *in vivo* clinical study assumed the use of thicker and stiffer PP catheters with a diameter of 1.74 mm and thinner and more flexible PE catheters with a diameter of 1.0 mm. Data from the literature show that rigid catheters are more convenient and easier to insert into the lumen of the uterus or fallopian tube, especially in cases of difficult ttransfers, eg, anatomical anomalies. However, it is associated with complications. The most common symptoms are bleeding, stimulation of the uterus or fallopian tube to contract, and mechanical damage to the endometrium or fallopian tube (6, 25). However, these complications do not have to reduce the effectiveness of transplantation and may even increase transfer efficiency (3, 5). There was also a reduction in the number of complications after transfer of embryos with hard catheters after the introduction of echogenic catheters under ultrasound control (3, 8).

In the case of PP catheters, due to the significant thickness of the catheter, stiffness, and lack of uniform surface structure, the assumed effectiveness was not achieved and the histopathological examination showed significant traumatization of the fallopian tubes. Complications were observed with the insertion of the PP catheter into the fallopian tube, the placement of the catheter along the natural course of the fallopian tube, and the deposition of embryos in its lumen. The catheter was inserted to a depth of 1.5-2 cm. There was a risk of damage to the fallopian tube and reduced effectiveness. This result was confirmed in a histopathological examination. After transplantation with a PP catheter, numerous and severe complications were found in one of the recovered fallopian tubes (Tab. 2 and Fig. 5). Inflammatory infiltrates, wall granulocytic infiltration, and wall granulation tissue clusters were found, with a visible defect in the wall of the fallopian tube after puncture, and also the presence of epidermal and fatty tissue fragments in the lumen of the fallopian tube. There were no purulent lesions. After the embryos were transferred with this catheter, 25% of the pregnancy was achieved (1/4).

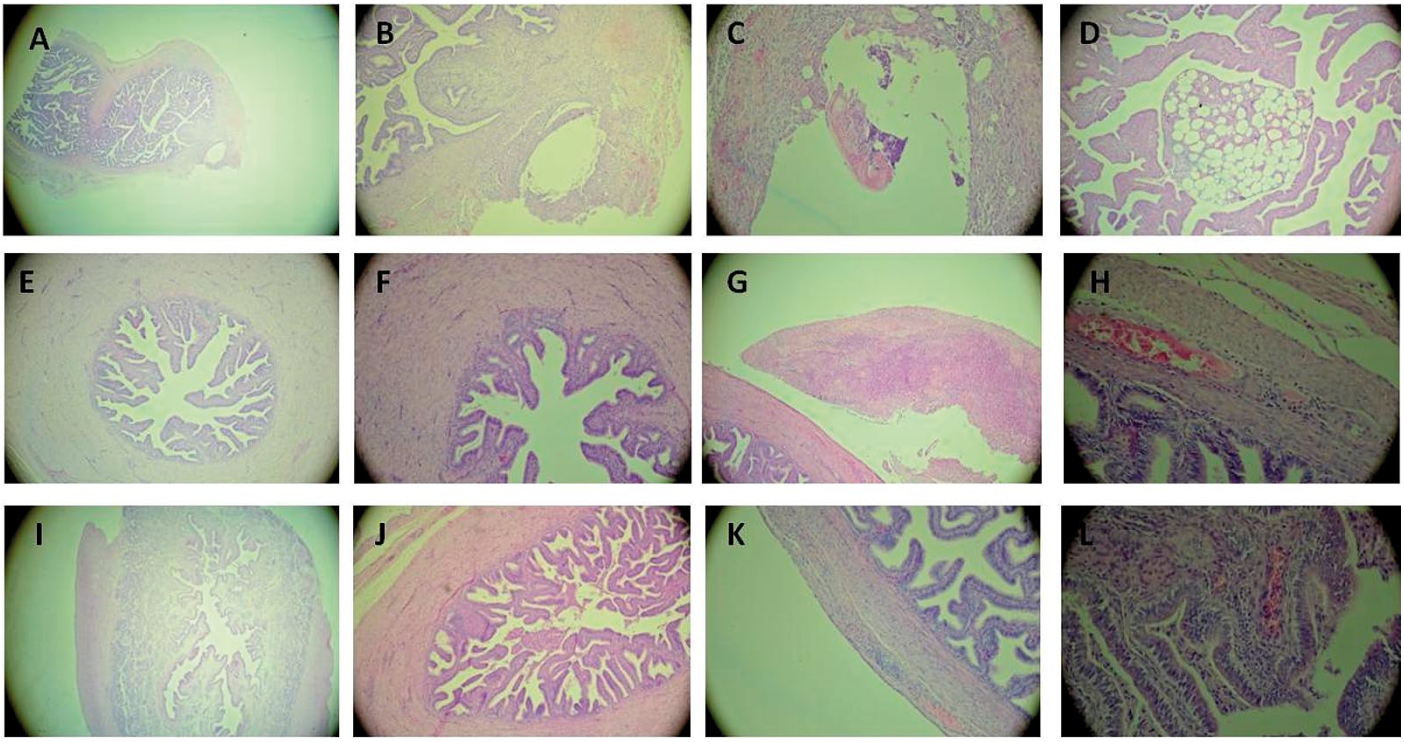

Fig. 5. Histological examination of the fallopian tubes. A-D – fallopian tube after transplantation with PP catheter; E-H – fallopian tube after transplantation with PE catheter; I-L – control fallopian tube. A – remnant of puncture in the fallopian tube, focus of acute inflammation with granulocytic infiltrate around the round defect (PP; 25 ×); B – focus of acute inflammation with granulocytic infiltrate around the circular defect (PP; 100 ×); C – clusters of nonspecific granulation tissue and mixed cell inflammatory infiltrates in the fallopian tube wall, a fragment of the epidermis within such foci (PP; 200 ×); D – in the lumen of the fallopian tube hyphae of inflamed adipose tissue (PP; 200 ×); E – normal fallopian tube, visible single granulocytes (PE; 100 ×); F – Oviduct in physiological norm, visible single granulocytes (PE; 200 ×); G – a small concentration of pus near the fallopian tube (PE; 100 ×); H – scanty granulocytic infiltrates in the fallopian tube wall and lumen granulocytes (PE; 400 ×); I – the structure of the mucosa and the lumen of the fallopian tube preserved, normal, sparse inflammatory infiltrates (Control; 200 ×); J – the structure of the fallopian tube is preserved, in the lumen of the fallopian tube a cluster of exfoliated epithelium (Control; 200 ×); K – minor inflammatory infiltrates (Control; 400 ×); L – very minor focus of inflammatory infiltrate (Control; 400 ×) (Nikon E600, Nikon, Japan)

Due to complications related to the insertion of the PP catheter into the fallopian tube and the low percentage of pregnancy, the effectiveness of this catheter was abandoned and the number of recipients was limited to a minimum of 5 animals. On the other hand, the high efficiency of embryo transplantation with these catheters would confirm that the production of embryo transfer catheters does not require materials with high quality features. Much greater effectiveness was obtained after embryo transplantation with a PE catheter. During embryo transfer with the PE catheter, no difficulties were observed with inserting the catheter into the lumen of the fallopian tube and depositing the embryos in the lumen of the fallopian tube. The small diameter, flexibility, and elasticity of this material allow the embryo-injured catheter to be inserted into the lumen of the fallopian tube at any depth along the natural course of the fallopian tube. With the PE catheter, the embryos were deposited about 1/3-1/2 of the length of the fallopian tube at a depth of 3-5 cm. After transfer of embryos with the PE catheter from 4 collected fallopian tubes, one showed a slight degree of change in mucosal structure, inflammatory infiltrates, granulocytic infiltration in the lumen and in the wall of the fallopian tube, granulocytic clusters in the vessels, and slight purulent changes in the fallopian tubes. Furthermore, in a control fallopian tube, slight inflammatory infiltrates were found (Fig. 5).

In terms of structure changes, the intensity of inflammatory infiltrates, and the preservation of the lumen of the fallopian tube, a significant similarity was found between the experimental and control groups. There were no statistically significant differences between post puncture and control fallopian tubes. The mild changes in the form of granulocytic infiltration in the wall and lumen of the fallopian tube were evaluated to not affect its functionality. The histopathological results obtained indicate minimal trauma, which does not affect the structure and physiology of the fallopian tubes. The use of this set allowed us to obtain a high efficiency of 70% of pregnancy (7/10). The efficiency of embryo transfer with the PE catheter was statistically higher compared to PP ($p < 0.05$).

The achieved method effectiveness is high (Tab. 3), and it is also higher compared to the results presented by other authors who described laparoscopic embryo transfer in pigs. The efficiency of PE catheter transfer is also comparable with the surgical method, the aver-

age efficiency of which is 50-60% (13, 18). The proposed method of embryo transfer was developed as an alternative to the classic surgical method, laprotomy. In the surgical method by laparotomy, significant traumatization and numerous complications limit its use (18, 21).

In the biological test, both PP and PP showed high biocompatibility. This was confirmed by examining the developmental potential of the transferred embryos after incubation under natural conditions (Tab. 4 and Fig. 6). After 5 days of incubation in the uterus, 12.5% of the 80 transferred embryos were washed, 10 embryos in the blastocyst stage (PP = 3, PE = 7). The developmental potential and quality of the embryos after *in vivo* culture after catheter transfer from PP and PE were similar (no statistically significant differences between the groups). Both groups had a similar number of nuclei in blastocysts and a very similar apoptotic index. Evaluation of embryo development potential allows the viability of embryos to be confirmed and is often used to assess the effectiveness of assisted reproductive methods (*in vitro* embryo breeding, *in vitro* fertilization or cloning) (10, 11). The blastocysts obtained, after 5-day incubation in the recipients' uterus, were characterized by a low apoptotic index with a small number of apoptotic cells. The values of the apoptotic index of leached blastocysts are comparable to those obtained under natural conditions *in vivo* (1.5-9.0) and significantly lower than those obtained after *in vitro* culture in 25-90% (9, 11). This indicates very good biological properties of PE and PP and a high biocompatibility of these materials. The developmental potential of embryos differs significantly between those obtained unde natural conditions and those obtained with assisted reproduction methods. A different degree of apoptosis was found in embryos obtained *in vivo* and *in vitro*. The differences are related to the process of DNA degradation and nuclear fragmentation during the preimplantation development of the embryos. These phenomena are observed in the earlier developmental stages of *in vitro* cultured embryos compared to *in vivo* conditions (32). The *in vitro* DNA degradation process occurs from stage 4-8 blastomeres and *in vivo* from the morula stage. Nuclear fragmentation is observed *in vitro* from the 8-16 blastomere stage, and *in vivo* – as before – from the morula stage (15).

As has been shown in the studies conducted, not all materials known and routinely used in medicine can be used in reproduction for embryo transplantation. To introduce new technologies and materials in reproduction, comprehensive research is required in terms of both materials, the possibility of producing catheters with the desired functional properties, and biological properties, including biocompatibility. In the case of PLA, it is not possible to form an appropriate catheter. In the case of PP, it is possible to produce catheters, but their thickness and the lack of a uniform surface structure exclude these angles. Of the tested materials, only PE has the appropriate material properties. An efficient method of laparoscopic embryo transfer in pigs has been developed using the developed PE catheter.

Tab. 3. Efficiency after laparoscopic transfer of pig embryo with PP and PE catheters

Material	Number of			
	transplanted embryos	recipients	transplanted embryos/ one recipient	pregnant recipients (%)
PE	274	10	30.2	7[a] (70%)
PP	121	4	27.4	1 (25%)
PLA	–	–	–	–
Total ET	395	14	28	8 (57%)
Histology/TUNEL				
PP	16	1	16	–
PE	64	4	16	–
Total Histology/TUNEL	80	5	16	–

Explanation: a – statistically significant difference PE vs PP, $p < 0.05$

Tab. 4. Assess the biocompatibility of PP and PE catheters based on the analysis of the developmental potential of transferred embryos

Group	Number of blastocysts	TUNEL		
		number of nuclei/ blastocyst	number of apoptotic cell/blastocyst	apoptotic index DCI (%)
In vivo culture of PP	3	60.00 ± 8.66	8.7 ± 2.08	14.7
In vivo culture of PE	7	54.86 ± 2.41	8.00 ± 1.91	14.5

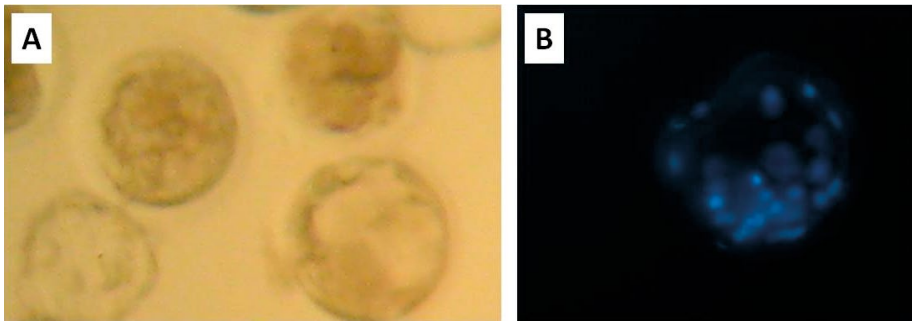

Fig. 6. Blastocysts obtained after 5-day incubation of transplanted embryos (A), TUNEL test of blastocyst development potential (B) (400 ×, Eclipse E600, Nikon, Japan)

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