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EXPERIMENTAL RATIONALE AND CLINICAL USE OF RADIOWAVE ENERGY, ANTI-ADHESION DRUGS, PLACENTA CRYOEXTRACT, RANGE OF PHYSIOTHERAPY MEASURES AND ASSISTED REPRODUCTIVE TECHNOLOGY IN THE RESTORATION OF REPRODUCTIVE FUNCTION OF PATIENTS WHO UNDERWENT OPERATIVE INTERVENTION CONCERNING TUBAL PREGNANCY

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Abstract

The aim: to increase the effectiveness of reproductive health restoration in women with progressive tubal pregnancy and develop new approaches to improving methods of restoring reproductive function in patients using endoscopy, anti-adhesive drugs, placental cryoextract, physiotherapy and ART. To substantiate the use of radio wave energy, anti-adhesive drugs, placental cryoextract, an experiment was performed on 196 mature female Wistar rats and 90 BALB / s mice. An analysis of clinical observations of 518 women. The control group included 60 healthy women, 458 patients with tubal pregnancy were divided into 2nd (152 patients), 3rd (154 patients) and 4th (152 patients) clinical groups in which different methods of treatment and rehabilitation of reproductive function were used.

It was proved that the use in the 2 clinical group patients PEO with CMC, streptodornase with streptokinase reduced the occurrence of adhesion at 1.6 times, use in patients 3 of the clinical group PEO with CMC, streptodornase with streptokinase and placenta cryoextract led to decrease occurrence of adhesions 2.2 times ($p < 0.05$), use in the

4th clinical group patients sodium hyaluronate, streptodornase with streptokinase and placenta cryoextract helped to reduce the occurrence of adhesions by 2.6 times ($p < 0.05$) compared with the detected intraoperatively adhesion process. The inclusion of ART methods in the treatment of patients who did not become pregnant contributed to the onset of uterine pregnancy in 64.9 - 75.7% of the patients under examination.

Key words: tubal pregnancy; reproductive function; radiowave energy; adhesion process; anti-adhesion barrier; physiotherapy treatment.

Introduction. The frequency of ectopic pregnancies in Ukraine is 15.4 per 1.000 pregnancies and it amounts 25-47% of all hospitalized in gynecological units. Tubal pregnancy (TP) accounts for 93-98.5% of all ectopic pregnancies. Among the causes that lead to TP, are previous inflammatory diseases of the female genital organs, the presence of adhesions in the pelvis developed after a previous laparotomy, etc. Previous reconstructive plastic surgery on the fallopian tubes leads to the development of TP in 15-27% of patients, and the use of assisted reproductive technologies (ART) causes ectopic embryo nidation in 1.5-2.1% of patients [1]. In this case, the reason for the increased risk of TP is not clear. Endometrial thickness > 12 mm before embryo transfer and the minimum number of transferred embryos are protective factors against TP occurrence [2].

During the treatment of TP patients, 61.3% undergo salpingectomy, 29.4% perform salpingotomy, and 4.6% undergo methotrexate treatment. Laparotomy access is performed by 46.7%, and laproscopic - 53.3% of surgical interventions in case of TP. After salpingectomy reperfusion and ischemic injuries of the ovary occur on the side of its performance. The normal menstrual cycle is restored in 68%, and pregnancy occurs in 20.0-42.5% of patients after unilateral salpingectomy.

Uterine tubes are permeable after conservative plastic surgery in 63-80% of patients. The adhesion process after laparotomy interventions develops in 94–100%, after laparoscopic interventions - in 81% without intraoperative use of anti-adhesive drugs. After operations to remove a fertilized egg from the tube without anti-adhesive drugs use, pregnancy occurs in 43.8–50.5% of patients, among whom 7–27% have a recurrent TP. The use of laparoscopic methods of TP treatment with preservation of the fallopian tube and the use of intraoperative lactate ringer, seprafilm, adept, mesogel, spraygel, surdjiflo matrix intraoperatively with the aim to prevent the development of adhesions reduces the incidence of adhesions to 31.3-40%; with that reproductive function recovers in 50–62.5% of patients in postoperative period, and reduced recurrence of TP is in 7–17% of patients [3]. The use of distreptase, laser therapy,

nonsteroidal anti-inflammatory drugs, combined oral contraceptives together with the listed anti-adhesive drugs, helps to restore reproductive function in 62.1–67.5% of patients and reduce the incidence of TP recurrence to 6.67–10.2% [4]. Later on in the postoperative period it is necessary to conduct a comprehensive rehabilitation therapy aimed at correcting hormonal status, normalization of the infectious index, contraception, normalization of contractile function of the operated fallopian tube, which increases treatment efficiency and reduces the level of recurrent TP [5]. Despite the use of modern laparoscopic methods of treatment, anti-adhesive drugs, physiotherapeutic methods of rehabilitation of TP patients, in 17-50% of them tubal-peritoneal infertility develops, which requires use of assisted reproductive treatment (ART) to restore the reproductive function. The average time to spontaneous pregnancy is 11.5 months, and after 24 months the risk of recurrent ectopic pregnancy increases [6].

The objective: to increase the effectiveness of reproductive health restoration in TP women during and after its laparoscopic treatment via experimental and clinical determination of the effects of different types of energy, anti-adhesive drugs, placental cryoextract (PC) and physiotherapy measures for tubal peritoneal infertility raise and recovery of fallopian tubes in the postoperative period, determine the degree of their impact on fertility and develop of new approaches to improving methods of restoring reproductive function of patients using endoscopy, anti-adhesive and biotechnological drugs (PC), physiotherapy and ART.

The experimental part of the work was performed on 196 adult female Wistar rats aged 5 months and 90 female BALB / s mice aged 3 months.

Studies in Wistar rats were performed To simulate the effect of diathermic, radiowave and surgical incision of the fallopian tubes with a scalpel in TP patients during their laparoscopic treatment and the occurrence of adhesions in the postoperative period the studies in Wistar rats were performed. To prevent adhesion in post-operative period

intraoperative prophylaxis with conventional methods, in particular, PEO with CMC was used. The experiment on female Wistar rats also simulated the effect of PC on the regeneration of fallopian tube tissues of TP women after surgery by tubotomy with removal of the fertilized egg. The effect of PC on the restoration of the histological structure of the uterine horns of the above experimental animals was studied. An assessment of the total impact of all these methods on the further implementation of the reproductive function of rats in the postoperative period.

Studies in BALB / s mice were performed for experimental substantiation the use of PC in TP women to restore reproductive function in the postoperative period by modeling

premature ovarian failure in experimental mice and subsequent restoration of their function due to the use of PC.

Materials and methods

The object of the 1st part of the experimental studies is represented by 196 adult female Wistar rats aged 5 months, average weight 230 g. All animals were divided into 6 study groups: 42 rats in the 1st, 2nd and 5th groups, 28 rats in the 3rd - 4th groups, 6 (control) group included 14 rats that did not undergo surgery. The operated fallopian tube was simulated in 182 animals of 1 - 5 groups. In 28 rats of the 1st group, diathermic energy with a power of 20 W was used for modeling at incision of the fallopian tube, suturing of the fallopian tube with mono AC 4.0 and the generally accepted method of prevention of adhesions development. In 28 rats of the 2nd group for modeling radiowave energy with a power of 20 W was used at incision of an oviduct, suturing of an oviduct by a mono ACI 4.0 and the generally accepted method of prevention of development of adhesive process. In 28 rats of the 3rd group for modeling radiowave energy with a power of 20 W was used at incision of an oviduct and anti-adhesive gel REO with CMC intraoperatively and PC i / m on 0.04 ml i / m once in 2 days 5 times for prevention of adhesions process development in the postoperative period. In 28 rats of the 4th group radiowave energy with a power of 20 W was used for oviduct incision, anti-adhesion gel sodium hyaluronate intraoperatively and PC i / m 0.04 ml i / m once in 2 days 5 times in the postoperative period. In 28 rats of the 5th (control) group, modeling of the operated fallopian tube was performed using a scalpel for oviduct incision, followed by suturing with mono AC 4.0, and the conventional method of prevention of adhesions was used. On the 7th day after the intervention, 70 animals (14 rats from the 1st, 2nd, 3rd, 4th, 5th groups) were withdrawn from the experiment with the study of the development of their adhesion process of varying severity and histological changes in oviducts. Subsequently, 70 rats (14 rats of the 1st, 2nd, 3rd, 4th, 5th groups) were mated with adult males and all animals were removed from the experiment on the 18th day after mating to study the onset of their pregnancy.

The object of the 2nd part of the experiment were 90 mice of BALB / s line with a weight of 20.1 ± 1.1 g aged 3 months, with a regular estrous cycle, which were divided into 3 groups of 30 animals: 1 - control group, 2nd - group with a model of premature ovarian failure (POF) without treatment, 3rd - group with a model of POF, treated with PC (0.01 g i/m, once per day for 5 days). POF was simulated by chemotherapy with busulfan 30 mg / kg and cyclophosphamide 200 mg / kg. POF treatment in 60 mice of groups 2 and 3 was started in 3 weeks after the beginning of chemotherapy, when all mice had no estrous cycle. We

studied the weight of mice, characteristics of the estrous cycle according to the colpocytogram, number of matings was assessed by detecting vaginal plugs. The terms of ovarian function and number of pregnancies were compared in all groups of animals.

The results of experimental studies were the basis for the development of therapeutic complex of advanced tubal pregnancy patients using only radio wave energy, REO with CMC, sodium hyaluronate during laparoscopic interventions, use of PC in combination with physiotherapy in postoperative period.

To address the goals and objectives, an analysis of clinical observations of 518 women was conducted. 1 clinical (control) group consisted of 60 apparently healthy women, and 458 progressive TP patients formed the groups under examination:

- 2nd group (152 patients). TP treatment with the use of laparoscopy - radio wave tubotomy, suturing of the fallopian tube wall with mono AC 4.0, use of PEO with CMC 40.0 ml intraoperatively for the prevention of adhesions and postoperative use of streptodornase and streptokinase, intrauterine enzyme electrophoresis, electrical stimulation of the fallopian tubes,

- 3rd (154 patients) interventions on the fallopian tubes using laparoscopic radiowave tubotomy, suturing of the fallopian tube wall with mono AC 4.0, using intraoperative PEO with CMC 40.0 ml intraoperatively to prevent the development of adhesions and in postoperative period use of streptodonase and streptokinase, PC №5, intrauterine enzyme electrophoresis, electrical stimulation of the fallopian tubes.

- 4th (152 patients) - laparoscopic radiowave tubotomy, suturing of the fallopian tube wall with mono AC 4.0, intraoperative administration of sodium hyaluronate 50.0 ml for the prevention of adhesions, streptodornase and streptokinase, PC №5, intrauterine enzymes electrophoresis, fallopian tubes stimuli in the postoperative period.

In 2 months after surgery, we performed metrosalpingography for all 458 patients. Patients with uterine obstruction according to MSG were referred for ART.

The results of experimental research and their discussion.

The result of the experiment on female Wistar rats

The use of diathermic energy for rats oviducts incision and use of conventional methods of adhesions prevention causes the development of adhesion of III and IV degree in the postoperative period, histic lack of oviducts which leads to significant decrease of embryos at 5.3 times.

The use of radio wave energy for the rats' oviducts incision, use of conventional methods of prevention of adhesions causes the development in the postoperative period

adhesions of the I and II degrees in most animals and a 3-fold reduction of the 3rd degree adhesions frequency, and the absence of the IV degree adhesion process, which increases the experimental animals fertility thrice compared with the corresponding indexes in the 1 group animals, but 1.8 times less than the corresponding indexes in the 6 (control) group of animals.

The use of radio wave energy for the incision of the oviducts of rats and the use of PEO with CMC in order to prevent the development of adhesions caused the development of adhesions only of the 1st degree in 42.9%, and its absence - in 57.1% of animals. It resulted in a significant increase in the number of embryos by 1.5 times compared with the corresponding indexes in rats of group 2 and the number of which did not differ significantly from the corresponding indicators in 6 (control) group of animals;

The use of radio wave energy for the incision of the oviducts of rats, PEO with CMC to prevent the development of adhesions and PC in the postoperative period caused a complete restoration of the oviduct structure, lack of adhesion in 64.3% of animals, adhesion of the first degree in 35.7% of animals, a significant increase (at 1.75 times) the number of embryos compared with the corresponding indicators in group 2 rats, the number of which significantly did not differ from the corresponding indexes in the 6 (control) group of animals.

The result of the experiment on 90 mice of the BALB / c line

In all animals of groups 1 and 2 after reproduction of the model of premature ovarian failure, the weight decreased sharply to 17.5 g with the following slow recovery.

At the same time, changes in the general condition were determined - hypodynamia, hairiness, turbidity of the eyes. The weight of the 2nd group animals corresponded to the indexes of the control group in 5 weeks and was 21 g, and at 8 weeks it was 22.1 g. In group 1, the animals regained weight to 21.1 g only in 8 weeks. At the same time, with the recovery of weight, the general condition and appearance of the animals improved. The dynamics of weight changes in groups 1, 2 and 3 is given in Fig.1.

When study the number of estrous cycles by vaginal swabs in all animals of the 3 (control) group a regular cycle was observed. In animals of groups 1 and 2 after reproduction of POF model, the absence of superficial epithelium in smears was observed, cyclicity appeared in 5 weeks after POF modelling in 20% of animals of group 1 and in 70% of the second. In 8 weeks, the restoration of the estrous cycle was observed in 50% of mice in group 1, which corresponds to the literature data on this model, while in the 2nd group animals its complete recovery was recorded (Fig. 2). Recovery of the estrous cycle correlates with the weight of mice. Thus, when comparing the weight of animals and the onset of a regular estrous cycle, it was noted that mice weighing less than 18 g often have its absence.

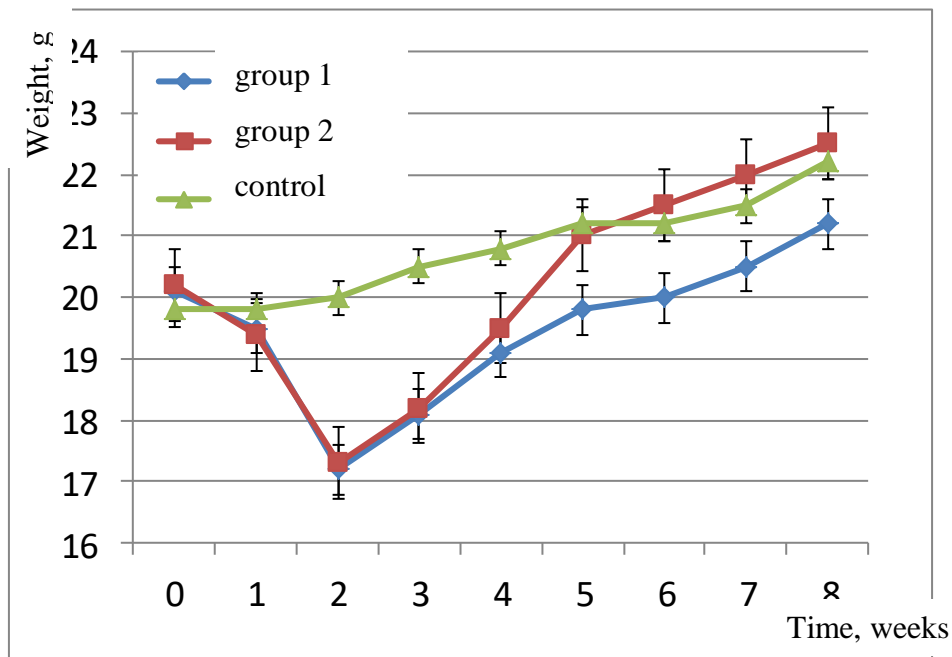


Fig. 1. Dynamics of experimental animals weight changes

The study of sexual function of animals of groups 1, 2 and 3 showed that the number of effective copulations in 1 group after reproduction of POF model was 30% in 8 weeks, which corresponds to the literature for the experimental model (Fig. 2). In the case of PC use for POF treatment, this index increased to 80%.

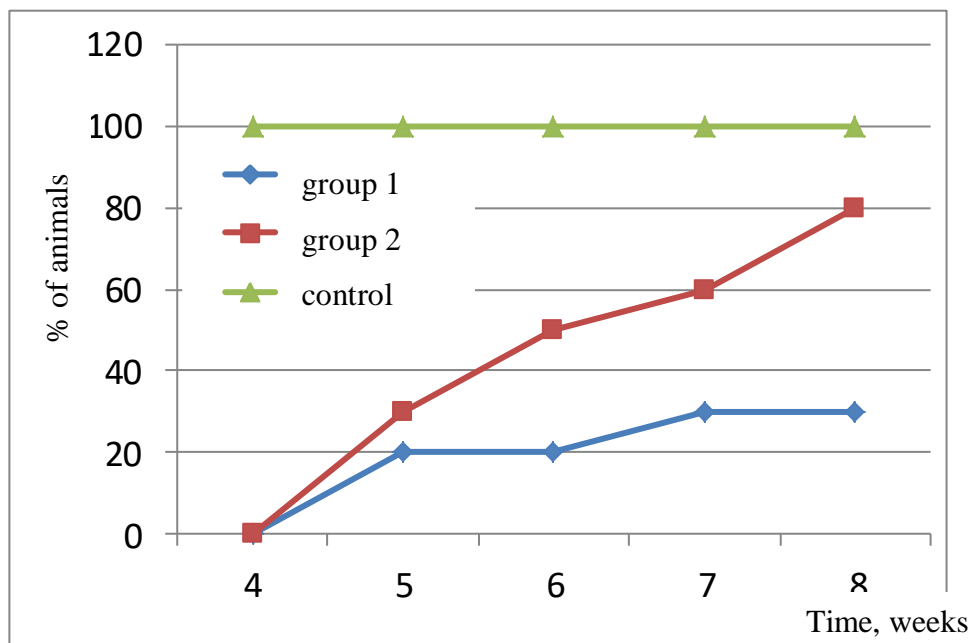


Fig. 2. Dynamics of recovery of sexual activity of experimental animals.

In the study of reproductive function, it was found that during the experiment in

females of the 3rd (control) group, pregnancy occurred in 90% of animals, an average 12 fetuses were born. However, in females of groups 1 and 2 during the 8-week follow-up none of pregnancies were registered, so the prospect of further research is to study the effect of cryopreserved placenta extract on the reproductive function of animals in the longer term, namely 12 and 16 weeks.

Morphological examination of experimental animals with simulated POF genitals was made in 12 weeks. It was found that the administration of cryopreserved placenta extract helps to restore the morphological structure of the uterus and partial restoration of ovarian structure with the appearance of follicle-like generative elements.

Intramuscular administration of cryopreserved placenta extract in mice with experimental POF helps to restore the weight of animals 1.6 times faster compared to the expected observation.

In 5 weeks after POF modeling, complete recovery of estrous cycles occurred in 20% of animals of group 1 and in 70% of the second group animals treated with PC.

When studying the sexual function of experimental animals, it was found that in 8 weeks the number of effective matings in group 1 was only 30%, while in group 2 with the administration of PC, this figure increased to 80%.

Thus, the results of experimental studies on 196 female Wistar rats and 90 BALB / c mice made it possible to compare the effectiveness of different types of energy and anti-adhesive agents, different methods of rehabilitation in the postoperative period in female rats with a model of the operated fallopian tube and mice with POF model.

Results of clinical trials and their discussion.

To determine the degree of influence of various anti-adhesive drugs in combination with PC on the development of adhesion in the postoperative period, we analyzed the development of adhesion in the postoperative period in 2, 3 and 4 clinical groups of patients according to MSG compared with its presence during laparoscopic intervention (Fig. 3, 4, 5).

The use of intraoperative PEO with CMC, suppositories with streptodornase and streptokinase reduced the occurrence of adhesions by 1.6 times in patients of clinical group 2 ($p < 0.05$), and the use of PEO with CMC intraoperatively, suppositories with streptodornase and streptokinase and PC at the postoperative period reduced the occurrence of adhesions by 2.2 times in patients of clinical group 3 ($p < 0.05$). That is, the inclusion of PC in the treatment of patients in whom an anti-adhesive agent was used - REO with CMC reduced the formation of adhesions in 1.4 times compared with the 2nd clinical group. The use of intraoperative sodium hyaluronate, suppositories with streptodornase and streptokinase and KP in the

postoperative period reduced the incidence of adhesions by 2.6 times in patients of clinical group 4 (p <0.05).

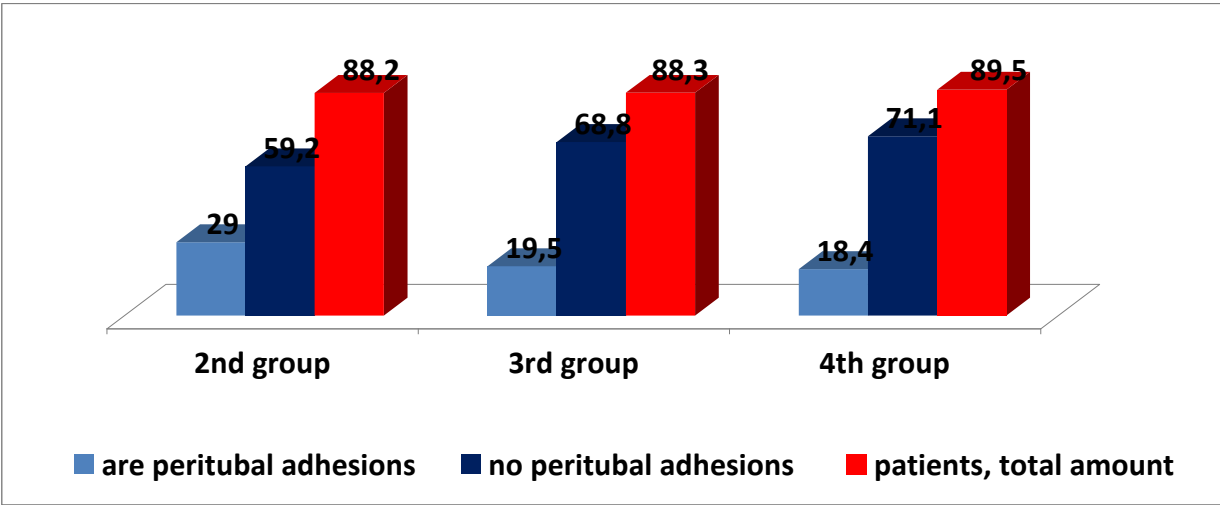


Fig.3. The condition of the fallopian tubes and the presence of adhesions.

Uterine tubes are patent,%

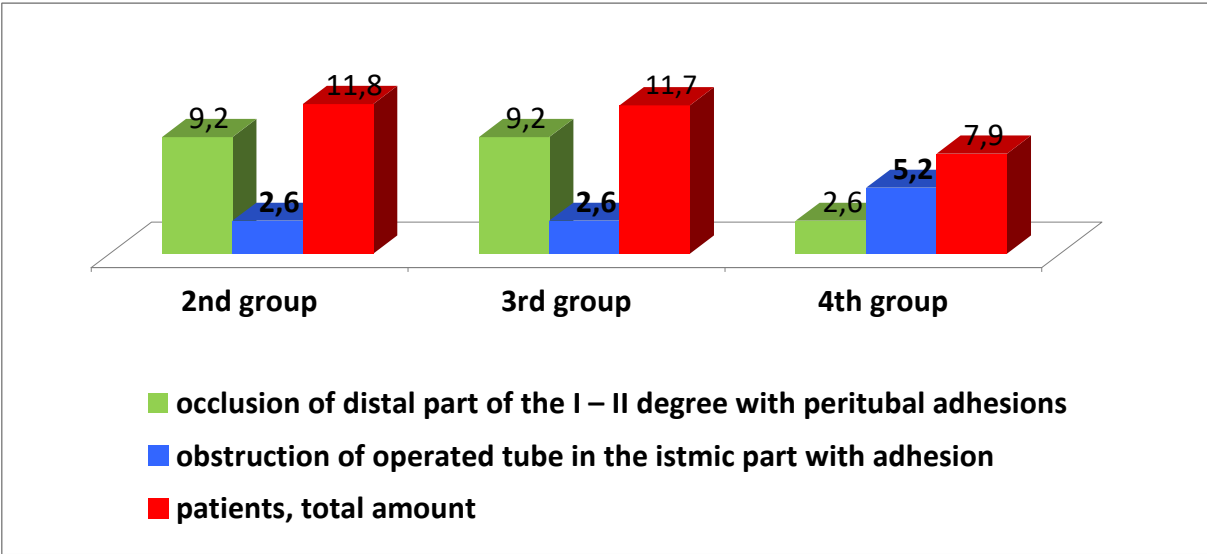


Fig.4. The condition of the fallopian tubes and the presence of adhesions

Obstruction of the fallopian tubes,%.

Thus, the inclusion of PC in the treatment of patients with the previous use of anti-adhesive agent - sodium hyaluronate reduced the formation of adhesions at 1.42 times compared with 2 clinical groups (p <0,05). The use of the above mentioned anti-adhesive drugs significantly reduced the percentage of adhesion compared to the use of

hydroperitoneum with dexamethasone in the 2nd group of patients with TP.

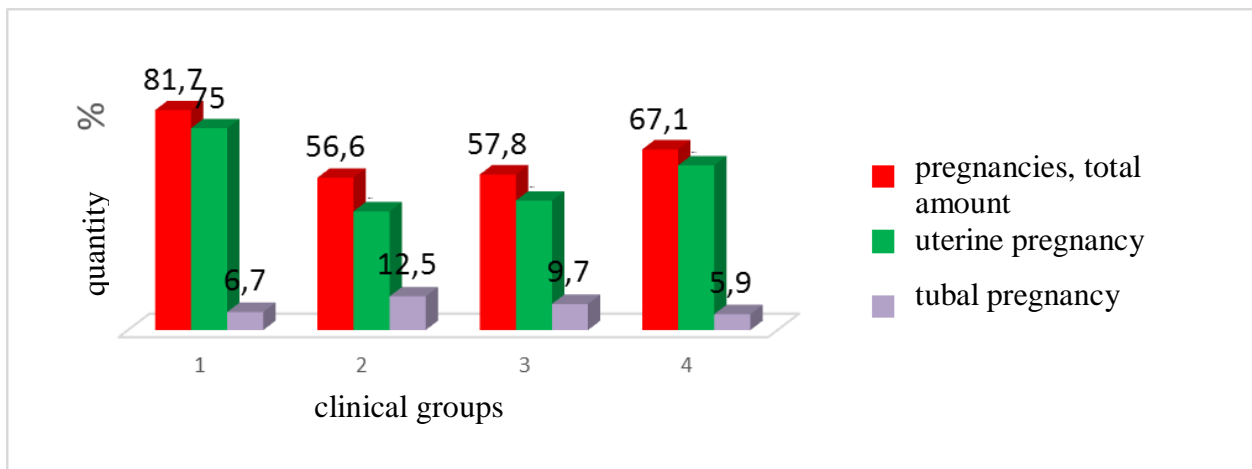


Fig.5. Pregnancy in the control group women, clinical groups - after laparoscopic treatment and rehabilitation

In total, reproductive function was restored after laparoscopic treatment and ART in 101 (66.4%) patients of clinical group 2, among whom uterine pregnancy occurred in 79 (52%), and recurrent TP - in 22 (14.5%) patients; in 117 (76%) patients of clinical group 3, uterine pregnancy occurred in 100 (64.9%), and TP - in 17 (11.0%) patients; in 126 (82.9%) patients of clinical group 4 uterine pregnancy occurred in 115 (75.7%), and TP - in 11 (7.2%) patients.

The results obtained as to fertility restoration in group 4 women significantly exceed the corresponding data of the 2nd clinical group patients, by 1.2 times ($p < 0.05$).

Based on the results of experimental and clinical studies, statistical processing of the results obtained we have made the following **conclusions**:

Use of diathermic energy for the oviducts incision in rats and the use of conventional methods of adhesion prevention leads to the development in the postoperative period III and IV degrees adhesions, histological failure of oviduct tissues, which in its turn, leads to oviducts tissues dehiscence. This resulted significant decrease at 4.5 times of the embryos amount after copulation compared with the same index in the control group of rats.

The use of radio wave energy for the oviducts incision in the 2nd group of rats and use of the generally accepted method of adhesion prevention causes the development in the postoperative period of the adhesion process of I and II degrees in most animals and a 3-fold reduction in occurrence of the 3 degree adhesions and the absence of the adhesion process of the IV degree, which, probably, increases the fertility of experimental animals by 3.0 times

compared with the corresponding data in group 1. When compare the corresponding data of groups 2 and 5, there is a significant 6 times reduction of the III degree adhesion process in group 2. The number of embryos in female rats of groups 2 and 5 was identical.

The use of radio wave energy for the incision of the oviducts of rats and the use of REO with CMC to prevent the development of adhesions, PC to restore the morphological structure of the oviducts led to the development of adhesions of the I degree only in 42.9% completely recovered oviduct's walls due to which the number of embryos significantly increases at 5 times compared with the same indexes in the 1 group rats. The amount of embryos in 3 and 4 female rats did not differ significantly from the corresponding indexes in group 6 (control). This may be a reason for the use of this scheme in clinical practice.

The use of PC in mice with a model of premature ovarian failure promotes recovery after 4 weeks of morphological structure of the uterus, partial recovery of ovarian structure with the occurrence of follicle-like structures. After 5 weeks recovery of sexual function in 70% of mice and after 8 weeks in 80% of animals took place.

The application of the developed methods of complex treatment, including ART in patients of the 2nd clinical group led to the onset of uterine pregnancy in 52.0% of patients ($p < 0.05$), tubal pregnancy - in 14.5% ($p > 0.05$) of patients, in 3 clinical group patients - in 64.9% ($p > 0.05$) and 11.0% ($p > 0.05$), in patients of the 4th clinical group - in 75.7% ($p > 0.05$) and 7.2 % ($p > 0.05$), respectively, compared with similar indexes of the control group women.

The use of radiowave energy in laparoscopic treatment, intraoperative use of PEO with CMC, streptodornase with streptokinase and physiotherapy in patients of the 2nd clinical group with TP without ART led to recurrent uterine pregnancy in 44.1%, which is statistically ($p < 0.05$) 1.7 times less, as well as the onset of recurrent TP in 12.5% of patients, which is equivocally ($p > 0.05$) 1.9 times more than the corresponding indicators of the control group women.

The use of radiowave energy in laparoscopic treatment, intraoperative use of PEO with CMC, PC, streptokinase with streptodornase and physiotherapy in TP patients of clinical group 3 ART led to the onset of subsequent uterine pregnancy in 48.1%, which statistically ($p < 0.05$) 1.6 times less and recurrent tubal pregnancy in 9.7% of patients, which is equivocally ($p > 0.05$) 1.4 times more than the corresponding data of women in the control group.

The use of radiowave energy in laparoscopic treatment, intraoperative use of sodium hyaluronate, PC, streptokinase with streptodornase and physiotherapy in patients with TP in

clinical group 4 without ART led to uterine pregnancy occurrence in 61.2% of cases, which is significantly ($p < 0.05$) 1.2 times less and recurrent TP - in 5.9% of patients, which is equivocally ($p > 0.05$) 1.1 times less than the corresponding indicators of the control group women.

The use of PEO with CMC intraoperatively in the postoperative period reduces the degree of adhesion after surgery by 1.6 times ($p < 0.05$) compared with its development after laparoscopic intervention with intraoperatively applied hydroperitoneum with dexamethasone.

The use of PEO with CMC intraoperatively and PC in the postoperative period reduces the occurrence of postoperative adhesions by 2.1 times ($p < 0.05$), and the use of sodium hyaluronate and PC in the corresponding periods reduces the occurrence of adhesions by 2.3 times ($p < 0.05$) compared with the same indicator in patients of clinical group 2.

The use of placental cryoextract in patients with unrealized reproductive function after laparoscopic treatment of TP in a set of rehabilitation measures causes a significant ($p < 0.05$) increase in 1.9 times the effectiveness of assisted reproductive technologies.

In order to optimize the method of restoring reproductive function in patients with tubal pregnancy, it is advisable to use laparoscopic organ-preserving treatment with intraoperative use of anti-adhesion agents - PEO with SMS or sodium hyaluronate, streptokinase with streptodornase, PC and complex physiotherapy treatment.

ART are advisable to use in patients with unrealized reproductive function after the complex treatment recommended.

The use of the above two-stage treatment contributes to the onset of uterine pregnancy in 64.9 - 75.7% of patients, which does not differ significantly from these indexes in the control group.

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