# A rabbit model for the evaluation of minimal access treatment of ectopic pregnancy in humans, using intrachorionic injection and local hyperthermia

L. W. POPP - R. GAETJE [*] - S. STATUS [*] - W. LIERSE † [**]

*Summary:* Rabbits at mid pregnancy were used as experimental models for investigating the feasibility of intrachorionic injection therapy in human ectopic pregnancy. Also, exposure of rabbit pregnancy to hyperthermia of 45°C was studied. The experiments were followed by serial serum progesterone levels and by light as well as electron microscopy.

Intrachorionic injection of different drugs led to demise in 92% of gestations. Application of hyperthermia to the pregnant rabbit uterus led in 94% to demise of gestations. Impairment of the reproductive functions after exposure did not occur.

Injection was more effective up to day 15 of pregnancy, while hyperthermia had better results in older gestations. Morphological reactions to injection of different drugs and to hyperthermia were similar. Irreversible damage to the uterine wall was not observed.

Intrachorionic injection of drugs may be a suitable treatment of human ectopic pregnancy. Application of hyperthermia may be a promising future therapy.

*Key words:* Ectopic pregnancy; Minimal access treatment; Intrachorionic injection; Local hyperthermia; Animal studies.

## INTRODUCTION

Ectopic pregnancy has a reported incidence of 1 to 2% of all pregnancies. The possible preventive benefit of early diagnosis and early treatment of ectopic pregnancy was already emphasized by Bothers in 1888 ([1]). Today, "early diagnosis" may be understood as detection of an unruptured ectopic pregnancy presenting no severe clinical symptoms which would necessitate immediate surgical intervention. Two weeks after the missed menses 98.1% of viable intrauterine, and 44.5% of ectopic pregnancies can be diagnosed by vaginal ultrasound examination (vaginosonography) ([2]). Therefore, vaginosonographic screening for ectopic pregnancy at 6 to 7 weeks post menstruation was proposed ([3, 4]). Detection of asymptomatic ectopic pregnancy may allow for therapeutic induction of demise of the pregnant products ([5, 6, 7]).

Clinic Dr. Guth, Juergensallee 46, D-2000 Hamburg 52, Germany

(*) Department of Obstetrics and Gynecology Christian Albrechts University Clinic, and Michaelis School of Midwifery, D-2300 Kiel, Germany

(**) Anatomical Institute, University of Hamburg, D-2000 Hamburg 20, Germany

Drugs interfering with trophoblastic growth have been administered systemically for treatment of ectopic pregnancy [8, 9]. Intrachorionic injection of drugs was carried out either laparoscopically or by guidance of vaginosonography [10, 11, 12, 13, 14]. There is no accepted recommendation regarding the appropriate substance, dosage, concentration, and volume for intrachorionic injection. We have, therefore, established an experimental rabbit model for in vivo evaluation of therapeutic injection of drugs and, also, of therapeutic exposure of the products of conception to hyperthermia. Application of hypothermia of 42 to 45° C has proved to be effective in cancer treatment [15]. Exposure of ectopic pregnancy to hyperthermia may induce demise of the products of conception as embryonic and trophoblastic tissues may be similarly prone to hyperthermia treatment as cancer cells. This new therapeutic possibility was proposed [6, 7] but has not yet been applied in humans.

## MATERIALS AND METHODS

*The rabbit model:* rabbits have a double uterus with separate ostia towards the vagina. A non-pregnant rabbit uterus is 7 to 8 cm long. The uterine wall is similar to that of the human tube and consists of a thick mucosa with longitudinal folds, a vascular lamina propria, an inner circular, and an outer longitudinal smooth muscle layer [16].

Duration of pregnancy in rabbits is 30 to 32 days [17]. An intrauterine rabbit gestation at mid pregnancy is in size and appearance similar to an unruptured human tubal pregnancy at 6 to 7 weeks post menstruation. The rabbit placenta is discoid and hemochorial as is the human placenta. The placenta is located on the mesometrium and consists of a thick basal decidua with uninucleate vesicular cells (Fig. 1A), an intermediate area of multinucleate vesicular cells, and the trophoblast which is labyrinthine in structure (Fig. 1B). The parietal decidua has a single layer of cylindrical cells which are partly ciliated, and a thin submucosa with uninucleate giant cells [18].

*Animal experiments:* in compliance with local laws, 61 white New Zealand rabbits bred specifically for laboratory studies, were used. The body weights of the rabbits ranged from 3.5 to 5.0 kg. They were 20 to 25 weeks old pregnant for the first time. Midline laparotomy was performed under general anesthesia between day 12 and 19 of pregnancy. A total of 503 intra-uterine gestations were found having outer diameters of 0.5 to 5.0 cm. The number of gestations in a single rabbit ranged from 1 to 17 with a mean of 8.5. All gestations in a rabbit were exposed to identical experiments.

The pregnant uteri of 3 rabbits were exstirpated to set a standard for the decline of serum progesterone levels after elimination of functional gestational tissue.

Possible ill-effects of the experiments caused by general anaesthesia, laparotomy with temporary external exposure of the pregnant uterus, and needle insertion into each gestation were studied in 3 control groups.

Injection of the following substances was performed into the rabbit gestations at laparotomy using an injection needle of 27 gauge:

- 0.9% NaCl solution, 0.5 ml and 1.0 ml;
- 0.1 mg methotrexate (0.04 ml) alone, and dissolved in 1 ml 0.9% NaCl solution;
- 0.05 IU ornipressin (POR 8®, Sandoz, Nuernberg) dissolved in 1 ml 0.9% NaCl solution;
- 70% ethanol, 1 ml;
- 7.45% KCl solution, 1 ml;
- 300 IU bovine thrombin (Topostasin®, Roche, Grenzach-Wyhlen), 1 ml;
- 1 µg prostaglandin $E_2$ (Nalador®, Schering, Berlin) dissolved in 1 ml 0.9% NaCl solution;
- $CO_2$ gas, 1 ml.

Application of hyperthermia was performed by submerging the surgically exposed, pregnant uterus in a bath of physiological saline solution set at 45° C.

The rabbits were sacrificed after littering, or on day 35 of pregnancy when no litter occurred. The uteri were removed and examined morphologically. Four rabbits of the hyperthermia groups were not sacrificed. They were mated again 2 months after successful experiments to evaluate the possible impairment of the reproductive functions which might have been caused by exposure of the uterus to 45° C.

*Hormonal assay:* progesterone serum levels were evaluated on alternate days using the Coat-A-Count® progesterone-radioimmunoassay (Diagnostic Products Corporation, Los Angeles).

*Morphological studies:* in 18 rabbits, morphologic studies of the gestations were performed at various intervals after the experiments. For light microscopy, the surgically removed gestations were fixed in formaldehyde and the specimens were stained with Hematoxylin Eosin. For electron microscopy, the minced tissues were
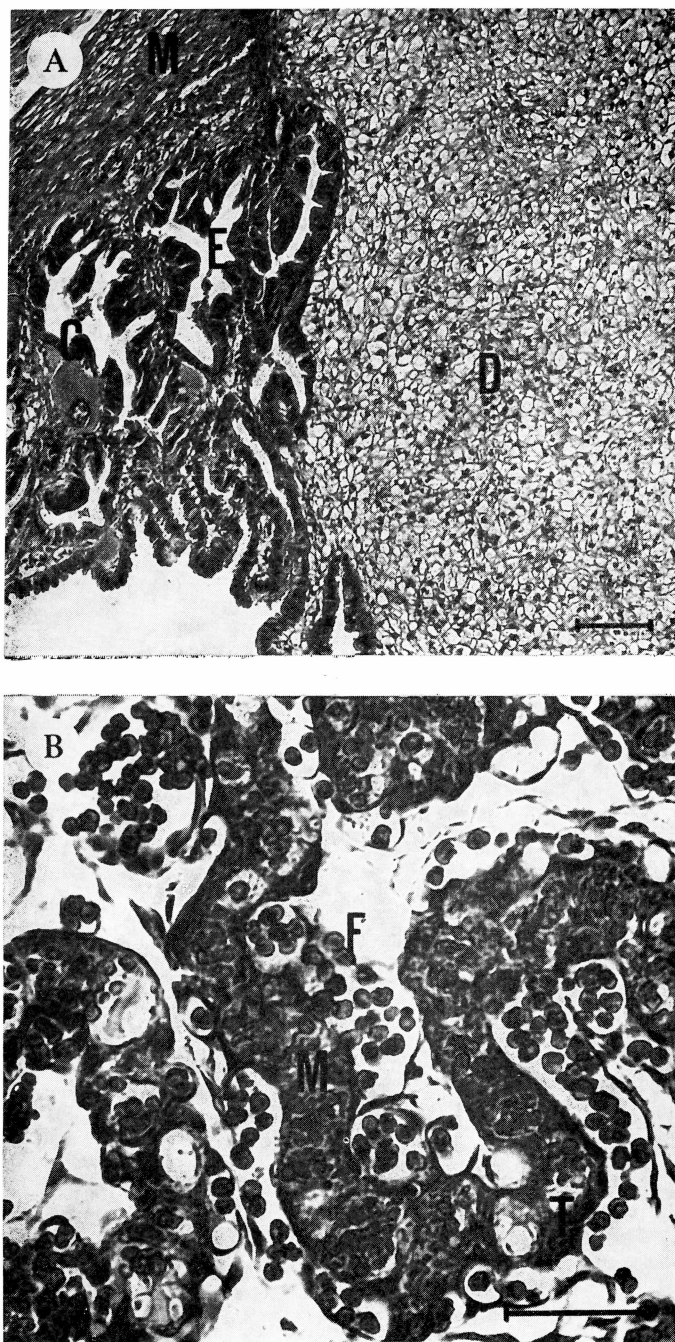
Fig. 1. — A) Decidua basalis on day 14 of pregnancy. D decidua, M muscle, G. giant cell, E uterine epithelium. — = 0.1 mm; B) Labyrinthine placenta on day 14 of pregnancy. M maternal red blood cells, T Trophoblast, F fetal red blood cells. — = 0.05 mm.
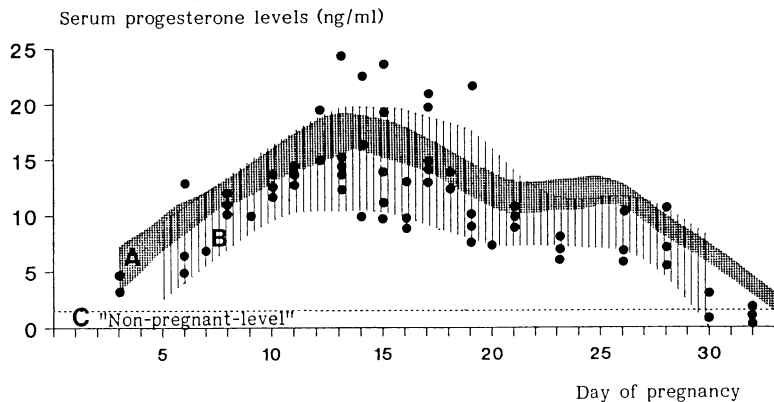
Fig. 2. — Serum progesterone levels in pregnant and non-pregnant rabbits. A) Rabbit pregnancy according to Lau *et al.* ([19]); B) Rabbit pregnancy according to own findings with ±s confidence area; C) « Non-pregnant-level ».

fixed using 2.5% glutaraldehyde in cacodylate buffer and postfixed with 2% osmium tetroxide. Combined uranyl acetate and lead citrate contrastation was used.

RESULTS

*Normal serum progesterone levels* (Fig. 2): 20 serum progesterone levels in 7 non-pregnant rabbits ranged from 0.1 to 3.1 ng/ml with an upper limit of confidence (± s) of 1.5 ng/ml. This value was accepted as the "non-pregnant level" of a rabbit. 62 serum progesterone levels in 9

pregnant rabbits between day 3 and 32 of pregnancy corresponded well to published data ([19]).

*Control groups* (Table 1): General anaesthesia was performed in 2 rabbits (No. 1, 2) on day 17 of pregnancy. Serum progesterone levels were not influenced, and litters occurred. Laparotomy with external exposure of the uterus revealed in 2 cases (No. 3, 5), done on days 12 and 17, normal serum progesterone levels and subsequent litters. In the third case (No. 4), done on day 12, decline of serum pro-

Table 1. — *Data of the control groups.*

| Group | Rabbit No. | INTERVENTION | | | LITTER | | Progesterone <1.5 ng/ml (days after intervention) |
|---|---|---|---|---|---|---|---|
| | | Day of pregnancy | Diameter of pregnancies (cm) | Number of pregnancies | Number of offspring | Day of litter | |
| General anaesthesia | 1 | 17 | ? | ? | 8 | 32 | 15 |
| | 2 | 17 | ? | ? | 5 | 30 | 13 |
| Laparotomy | 3 | 12 | 0.5 - 2.2 | 17 | 7 | 31 | >18 |
| | 4 | 12 | 1.6 - 2.1 | 9 | – | -- | 8 |
| | 5 | 17 | 2.5 | 10 | 5 | 32 | 14 |
| Needletick | 6 | 12 | 1.9 - 2.4 | 14 | – | – | 2 |
| | 7 | 13 | 1.7 | 13 | – | – | 8 |
| | 8 | 17 | 1.3 - 3.0 | 14 | 4 | 34 | 11 |
| | 9 | 17 | 2.0 - 3.0 | 8 | 7 | 33 | 11 |

Table 2. — *Data of the hysterectomy standard and the successful experimental groups.*

| Group | Rabbit No. | INTERVENTION | | | LITTER | | Progesterone <1.5 ng/ml (days after intervention) |
|---|---|---|---|---|---|---|---|
| | | Day of pregnancy | Diameter of pregnancies (cm) | Number of pregnancies | Number of offspring | Day of litter | |
| Hysterectomy | 10 | 13 | 1.1 | 1 | – | – | 3 |
| | 11 | 16 | 2.5 | 2 | – | – | 3 |
| | 12 | 17 | 3.0 | 6 | – | – | 2 |
| 0.1 mg methotrexat in 1 ml NaCl (0.9%) | 13 | 12 | 1.8 | 12 | – | – | 8 |
| | 14 | 12 | 1.2 - 2.0 | 10 | – | – | 5 |
| | 15 | 13 | 0.8 - 2.5 | 11 | – | – | 4 |
| 1 ml KCl (7.45%) | 16 | 16 | 2.5 | 5 | – | – | 3 |
| | 17 | 16 | 2.5 | 3 | – | – | 2 |
| 1 ml ethanol (70%) | 18 | 12 | 1.5 - 2.5 | 6 | – | – | 6 |
| | 19 | 12 | 2.5 | 4 | – | – | 4 |
| | 20 | 13 | 1.5 | 4 | – | – | 5 |
| 1 ml thrombin (300 IU) | 21 | 15 | 2.0 | 11 | – | – | 8 |
| | 22 | 15 | 2.5 | 6 | – | – | 5 |
| | 23 | 15 | 2.0 | 10 | – | – | 5 |
| 1 µg PG E$_2$ in 1 ml NaCl (0.9%) | 24 | 15 | 2.5 | 6 | – | – | 3 |

gesterone to the non-pregnant level occured within 8 days with no subsequent litter. A needle stick into all gestations revealed in 2 rabbits (No. 6, 7), operated on days 12 and 13 of pregnancy, decline of serum progesterone to non-pregnant level within 2 and 8 days respectively, and no litters. In two other cases (No. 8, 9), done on day 17, litters occured.

*Hysterectomy group and successful injection groups* (Table 2): Decline of serum progesterone to the "non pregnant level" of 1.5 ng/ml took 2 to 3 days after hysterectomies done in 3 rabbits (No. 10-12) on days 13, 16, and 17 of pregnancy. Successful induction of demise of all pregnancies in a rabbit was accepted when decline of serum progesterone to the non-pregnant level took not more than 8 days. This was observed in all rabits of an experimental group after injections of 0.1 mg methotrexate dissolved in 1 ml 0.9% NaCl solution (No. 13-15), 1 ml KCl solution (7.45%) (No. 16, 17), 1 ml ethanol (70%) (No. 18-20), 1 ml bovine thrombin (300 I.U.) (No. 21-24), and 1µg prostaglandin E$_2$ dissolved in 1 ml 0.9% NaCl solution (No. 24). The interventions were performed between days 12 and 16 of pregnancy.

*Partly successful and unsuccessful injectio groups* (Table 3): Injection of 1 ml physiological saline solution met the standard of a successful experiment in 4 rabbits (No. 25-28) when interventions were performed on day 15 of pregnancy. The same experiment in 2 other rabbits (No. 29, 30), done on day 16, was not successful.

Injection of 0.5 ml of physiological saline solution into 3 gestations in a rabbit (No. 31) on day 17 was unsuccessful as a litter with 3 offspring occurred on day 34 of pregnancy. Injection of 0.1 mg methotrexate (0.04 ml) on day 14 of pregnancy was successful in 1 rabbit (No.

Table 3. — *Data of the partly successful and unsuccessful experimental groups.*

| Group | Rabbit No. | INTERVENTION | | | LITTER | | Progesterone <1.5 ng/ml (days after intervention) |
|---|---|---|---|---|---|---|---|
| | | Day of pregnancy | Diameter of pregnancies (cm) | Number of pregnancies | Number of offspring | Day of litter | |
| 1 ml NaCl solution (0.9%) | 25 | 15 | 0.5 - 2.0 | 5 | – | – | 7 |
| | 26 | 15 | 1.5 - 2.5 | 8 | – | – | 4 |
| | 27 | 15 | 2.0 | 3 | – | – | 4 |
| | 28 | 15 | 2.0 - 2.5 | 12 | – | – | 2 |
| | 29 | 16 | 2.0 - 3.0 | 7 | – | – | 15 |
| | 30 | 16 | ? | 8 | 3 | 33 | 13 |
| 0.5 m NaCl solution | 31 | 17 | 2.6 | 3 | 3 | 34 | 14 |
| 0.1 mg methotrexate (0.05 ml) | 32 | 14 | 1.6 - 2.1 | 11 | – | – | 7 |
| | 33 | 17 | 3.0 - 3.5 | 5 | 2 | 33 | 14 |
| | 34 | 19 | 4.0 - 5.0 | 10 | 3 | 33 | 13 |
| 0.05 IU ornipressin in 1 ml NaCl (0.9%) | 35 | 13 | 1.7 | 9 | – | – | 4 |
| | 36 | 16 | ? | 13 | – | – | 2 |
| | 37 | 16 | ? | 12 | – | – | >15 |
| | 38 | 16 | ? | 14 | 1 | 30 | 15 |
| | 39 | 19 | 4.0 | 11 | 4 | 31 | >12 |
| 1 ml CO₂-Gas | 40 | 19 | 4.5 | 1 | – | – | 12 |
| | 41 | 19 | 3.5 - 4.5 | 11 | 3 | 33 | 9 |

32). The same experiment was unsuccessful in 2 other rabbits (No. 33, 34) with interventions on day 17 and 19 of pregnancy. Injection of 0.05 I.U. ornipressin dissolved in 1 ml physiological saline solution revealed 2 successful cases (No. 35, 36) when the interventions were done on day 13 and 16, and 3 unsuccessful cases (No. 37-39), which were injected on day 16 and 19 of pregnancy.

Injection of 1 ml carbon dioxide gas into the uterine gestations of 2 rabbits on day 19 of pregnancy (No. 40, 41) was unsuccessful.

*Hyperthermia groups* (Table 4): Application of hyperthermia of 45° C to the pregnant uteri for 20 minutes was unsuccessful on day 13 of pregnancy in 2 rabbits (No. 42, 43), and successful on day 16 in another 2 cases (No. 44, 45). When using 10 minutes exposure time, 3 experiments (No. 46, 47, 49), done on

days 12 and 16 of pregnancy were unsuccessful, and another 3 (No. 48, 50, 51) were successful.

2 months after successful exposure of their pregnant uteri to hyperthermia, 4 rabbits were mated again. All of them concieved at the first attempt and the courses of their pregnancies were normal.

*Morphological findings*: The uterine wall was intact in all specimens. Exposure to hyperthermia caused slight hydropic swelling of the muscularis layer. After injection of KCl solution, the parietal decidua had necrotic defects and desquamations, which were repaired after 5 days.

The uninucleate cells of the basal decidua were not altered half an hour after injections. After exposure to hyperthermia small necrotic areas were seen. In electrone microscopy, cytoplasmatic swelling of the uninucleate cells was observed in all groups (Fig. 3a). The multinucleate

Table 4. — *Data of the hyperthermia groups.*

| Group | Rabbit No. | INTERVENTION | | | LITTER | | Progesterone <1.5 ng/ml (days after intervention) |
|---|---|---|---|---|---|---|---|
| | | Day of pregnancy | Diameter of pregnancies (cm) | Number of pregnancies | Number of off-spring | Day of litter | |
| Hyperthermia (45° C, 20 min) | 42 | 13 | 1.7 | 13 | 1 | 33 | >16 |
| | 43 | 13 | 1.0 - 1.7 | 13 | – | – | 12 |
| | 44 | 16 | 2.5 | 17 | – | – | 7 |
| | 45 | 16 | 1.5 - 3.0 | 5 | – | – | 3 |
| Hyperthermia (45° C, 10 min) | 46 | 12 | 1.8 | 10 | 5 | 32 | >18 |
| | 47 | 12 | 1.5 | 8 | – | – | >20 |
| | 48 | 12 | 1.4 - 1.9 | 2 | – | – | 8 |
| | 49 | 15 | ? | 9 | – | – | 13 |
| | 50 | 15 | ? | 8 | – | – | 3 |
| | 51 | 16 | ? | 12 | – | – | 1 |

cell layer and the trophoblast were not affected.

One to 10 days after an intervention, in all groups necrosis was found in the upper half of the basal decidua (Fig. 3b), the multinucleate cell layer, and the trophoblast. The lower part of the basal decidua was microscopically intact. Few leucocytes and no macrophages were found around the necrotic areas. In the hyperthermia group additional bleeding was observed in the trophoblast.

After sacrificing the rabbits, focal infiltrations of leucocytes were seen in the stroma of the endometrium. Uninucleate basal decidua cells, giant cells, and macrophages were sporadically found in the submucosa. The myometrium was intact. The serosa was smooth and there was no evidence of inflammation or adhesions.

DISCUSSION

Rabbits at mid-pregnancy are suitable experimental models for the evaluation of local treatment of ectopic pregnancy in humans. A characteristic pattern of serum progesterone levels correlates to intact pregnancy, and decline of serum progesterone levels below 1.5 ng/ml ("non-pregnant level") may be used as a parameter for demise of all gestations in a rabbit.

Gestational age plays a role in the induction of pregnancy demise in rabbits. External exposure of the pregnant uterus, a needle stick, and injections into the gestations were successful up to day 15 of pregnancy, regardless of the substances used. After that time, the majority of injection experiments were unsuccessful. Contrastingly, gestations undergoing application of hyperthermia on day 12 and 13 of pregnancy were less prone to pregnancy demise as compared to those on day 15 and 16 (Table 4). This phenomenon might be explained by the primary manifestation of the hyperthermic noxis in the basal decidua; whereas after injections, the trophoblast was affected first. Also, the injected volume may be of importance as induction of pregnancy demise was less successful when less than 1 ml was used.

After induction of pregnancy demise by injection morphological findings were similar for all drugs used. It remains, therefore, an open question, which substance may be best for intrachorionic injection therapy in human ectopic pregnancy. According to our data and to clinical practicability, there might be a tendency of
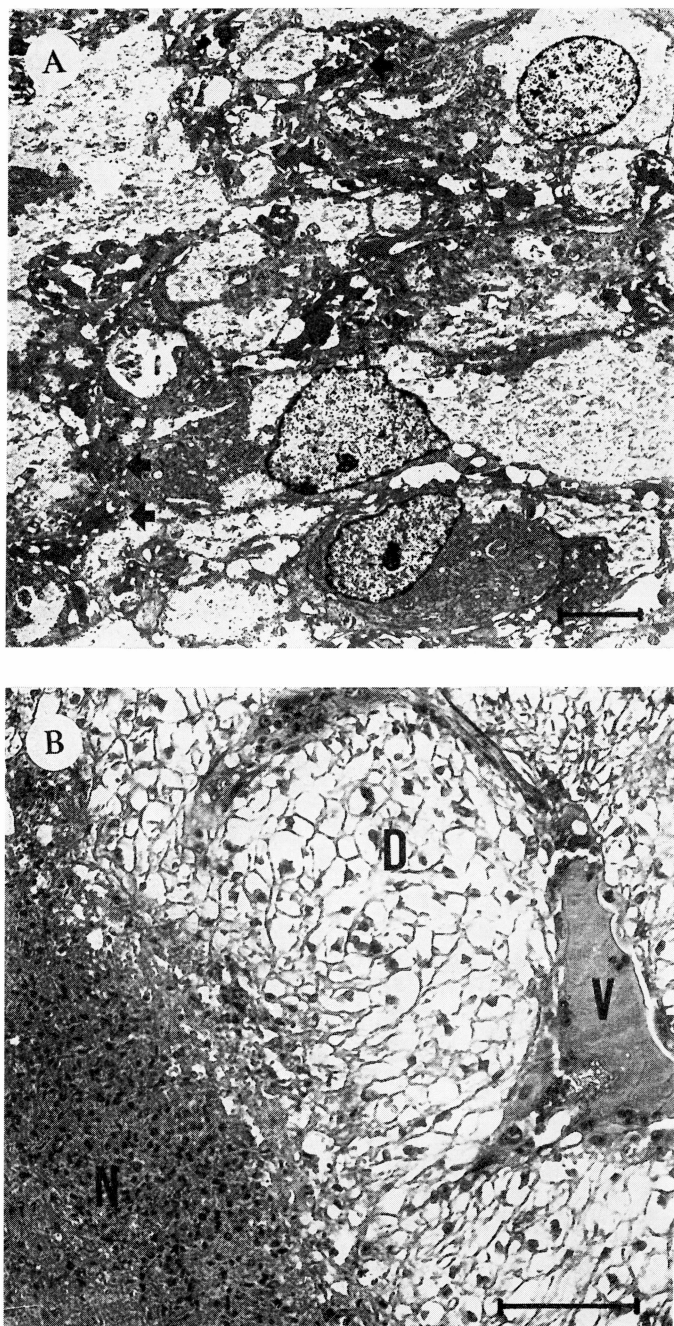
Fig. 3. — A) Basal decidua in electron microscopy half an hour after injection showing cytoplasmatic swelling (➤). — = 1 μm; B) Abrupt transition from necrotic to intact basal decidua one day after injection. N necrosis, D decidua, V maternal blood vessel. — = 0.1 mm.

choosing 1 ml of KCl solution (7.45%), as the results on day 16 of rabbit pregnancy (No. 16, 17, Table 2) were identical to the hysterectomy standard.

Induction of pregnancy demise in rabbits led to progressive necrosi of the products of conception. In the absence of invasion of larger numbers of leucocytes and macrophages around the necrotic areas, subsequent expulsion of the necrotic tissues can be assumed. In demised ampullary pregnancy in human, tubal abortion and subsequent intraabdominal absorption can be exprected as the parallel event. In other locations of ectopic pregnancy, persistance of the demised products of conception for longer periods of time may be expected. This corresponds with unpublished clinical experience of several investigators, who have reported symptoms of tubal abortion some days after injection therapy of tubal pregnancy, and long time persistance of demised pregnant products e.g. after injection of an intramural pregnancy.

Consequently, induction of pregnancy demise in human ectopic pregnancy should only be attempted at an early stage. We have set our limit to 2 cm chorionic cavity diameter which, in viable ectopic pregnancy, corresponds to 7 weeks post menstruation. Tubal abortion of small necrotic remnants may cause less trauma than operative removal. This may result in higher rates of subsequent intrauterine pregnancies and smaller rates of recurrencies. In intramural pregnancies, small remnants in the myometrium may be the better therapeutic choice when compared to the trauma of surgical intervention.

The prompt occurance of normal pregnancies after hyperthermia experiments gave striking biological proof of the subsequent integrity of reproductive functions in rabbits. Vaginosonographically guided application of hyperthermia to an early detected human ectopic pregnancy may, therefore, be a promising future therapy.

## CONCLUSION

Intrachorionic injection of drugs may be a suitable treatment of human ectopic pregnancy. Application of hyperthermia may be promising future therapy.

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Address reprints requests to:

L. W. POPP
Juergensallee 44
22609 Hamburg, Germany