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Ovarian function after salpingostomy following mechanically produced hydrosalpinx in rabbits

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Summary

The discrepancy between pregnancy and patency rates after salpingostomy is explained largely by the residual functional damage to both the mucosa and smooth muscles of the fallopian tube. The function of the ovary after salpingostomy has not been extensively studied. Hydrosalpinx was mechanically induced by double clip application in thirty adult female New Zealand white rabbits. After varying periods of hydrosalpinx formation, ampullary-isthmic anastomosis and cuff-salpingostomy were carried out using full microsurgical techniques. The animals were mated 1 week after surgery. Ten days after mating, laparotomy was performed. The contralateral oviducts and oviducts from unoperated animals served as controls 1 and 2 respectively. Corpora lutea counts and ovarian venous estradiol 17_B (E_2) and progesterone (P) levels were measured to assess ovarian function. The mean values (± s.e.) for corpora lutea count on Control 1, Control 2 and operated sides were 6.46 ± 0.43, 5.69 ± 0.44 and 4.03 ± 0.32 respectively. The mean values for E_2 and P were 159.0 ± 17.43 and 281.769 ± 13.58; 133.75 ± 18.45 and 265.89 ± 18.12; 128.57 ± 14.66 and 206.103 ± 13.296 respectively. Only the differences between the mean number of corpora lutea and mean progesterone were statistically significant ($P < 0.001$) ($0.02 > P > 0.01$) respectively. There was no relationship between the number of corpora lutea and the levels of E_2 and P. We conclude from this study that ovarian function is impaired after salpingostomy in the rabbit. The possible mechanism is discussed. We feel that this may have some relevance to humans.

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Résumé

La disparité constatée, après la salpingostomie, entre les taux de grossesse et ceux de la fonction tubaire, est due essentiellement à la pathologie fonctionnelle residuelle de la muqueuse et des muscles lisses de la trompe de Fallope. La fonction ovarienne suivant une salpingostomie a fait jusqu'ici l'objet de peu d'études. Au moyen de la technique de la double pince, l'hydrosalpinx a été inoculé à trente lapines blanches adultes de type néo-zélandais. Après des durées variables d'hydrosalpingite, on a effectué, à l'aide de techniques microchirurgicales, l'anastomose ampullo-isthmique et la salpingostomie ampullaire. Une semaine après l'intervention, les animaux étaient mis aux mâles et subissaient la laparotomie à 10 jours de grossesse. Les oviductes contralatéraux ont été utilisés comme témoin 1, et ceux obtenus d'animaux non opérés comme témoin 2. Afin d'évaluer la fonction ovarienne, on a noté la quantité de corps jaune, le volume d'oestradiol veineux ovarien 17_B (E_2) ainsi que la concentration de progesterone (P). Les valeurs moyennes (± s.e.) de corps jaune ont été de 6.46 ± 0.43 pour le témoin 1; de 5.69 ± 0.44 pour le témoin 2 et de 4.03 ± 0.32 pour les côtés non opérés. Les valeurs moyennes de E_2 et de P ont été de 159.0 ± 17.43 et de 281.769 ± 13.58 pour le témoin 1; de 133.75 ± 18.45 et de 265.89 ± 18.12 pour le témoin 2 et de 128.57 ± 14.66 et de 206.103 ± 13.296 pour les côtés non opérés. Seules les différences entre la valeur moyenne

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de corps jaune et la progestérone ont eu une importance statistique quelconque. Il n'y a pas eu d'affinité entre la quantité de corps jaune et les volumes de E_2 et de P. De la présente étude nous tirons la conclusion que, suite a la salpingostomie chez le lapin, la fonction ovarienne diminue. Nous discutons du processus, lequel nous semble pouvoir s'appliquer aux humains.

Introduction

With the introduction of microsurgical techniques for tubal anastomosis to reverse sterilization, evidence began to accumulate that surgical modification of the tube may be associated with decreased ovarian function. Berger, Radwanska and Hammond (1978), showed that the mean luteal plasma progesterone in eight women requesting sterilization was significantly lower than in normal female controls. Radwanska, Berger and Hammond (1979) confirmed these findings. They showed that forty women with normal menstrual periods who requested reversal of sterilization had a mean luteal progesterone level which was significantly lower than levels in a control group of twenty-four unsterilized women. Only a single estimate of plasma progesterone was made in this study. Donnez, Wanters and Thomas (1981), compared sterilized women with controls using various methods. They noted that plasma progesterone was significantly lower in women sterilized by electrocoagulation or tubal ligation, compared with unsterilized women or women sterilized by clips. However, Corson *et al.* (1981) found no evidence of depression of mid-luteal progesterone levels in women after hysterectomy or tubal sterilization by various methods.

Recent animal studies raise the possibility that surgical modification of the fallopian tube is associated with suppressed ovarian function. Koninckx *et al.* (1980), showed that the number of corpora lutea was significantly lower after transection and anastomosis of the ampulla and the ampullo-isthmic junction but not after transection and anastomosis of the isthmus of the rabbit oviduct. In a similar study, McComb, Bourdage & Halbert (1983), found that there were fewer ovulations in the ipsilateral ovary after fimbriectomy with ampullary resection in rabbits. Both these studies suggest that interference with the neuro-vascular connection between the fimbria and the ovary may be responsible for the partial suppression of ovarian function. Beyth and Winston (1981) noted that there were fewer ovulation sites after unilateral fimbriectomy on the operated compared to the control side in a series of sixteen rabbits. They offered no explanation for this statistically significant phenomenon. In all these animal studies, surgical modification of the ampulla and/or fimbria seem to be a common factor associated with lowered ovarian function. Hydrosalpinx is a disease of the ampulla and its therapy: salpingostomy, involves surgical modification of both the ampulla and the fimbria. This study was carried out to assess the effect of hydrosalpinx and its surgical therapy: salpingostomy, on ovarian function in the rabbit experimental model.

Materials and methods

Animals

Thirty New Zealand white rabbits weighing 2.5–3 kg were used in the study. They were isolated for 3 weeks before use. They were fed on rabbit chow and given water *ad libitum*. They were allowed 12 h of alternating light and darkness.

Induction of hydrosalpinges

Laparotomy was done under general anaesthesia. The fallopian tube on one side was identified and one stainless ligating clip (Ligaclip-Ethicon Ltd) was applied across the fimbrial end of the ampulla. Another clip was applied across the ampullary-isthmic junction. Care was taken to avoid damage to the blood vessels supplying the oviduct. The duration of clip application varied from 8 to 52 weeks.

After this period, salpingostomy and ampullary-isthmic anastomosis were carried out. The results of surgery have been reported elsewhere (Otubu & Winston, 1983). The contralateral fallopian tubes served as the first control (control 1). A second group of controls consisted of eight normal unoperated animals (control 2). All animals were mated with a fertile buck and subjected to laparotomy on the

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tenth day after mating. They were given systemic heparin about 30 min before laparotomy. A midline incision was made and each ovary exposed. The number of corpora lutea and number of implantation sites were recorded.

The exposed intestines were wrapped in gauze soaked in warm sodium lactate and tucked back under the abdominal wall. The tributary of the ovarian vein which drains the oviduct and part of the uterus was clamped. The ovarian vein was exposed and isolated by careful dissection. This was particularly necessary in animals in which hydrosalpinx had been present for long periods, as excess fat tended to obscure the vessels. A 25-gauge needle attached to a heparinized 5-ml syringe, was carefully guided into the ovarian vein and 5 ml of blood was drawn. This blood was centrifuged and the plasma was removed and stored at −20°C for subsequent steroid assay.

Radioimmunoassay (RIA) of estradiol 17_B and progesterone

Plasma sample volume used for extraction varied from 0.1 ml to 0.5 ml. Samples were run in duplicate. Estradiol 17_B and progesterone samples were extracted once with 10 volumes of chilled diethyl ether (Mallinrodt Anaesthesia Grade). Snap freezing was done in a dry ice–acetone bath. The ether phase was then evaporated to dryness under a slow nitrogen stream. For recovery, an aliquot of plasma was incubated with labelled steroids (1000 cpm) as 1, 2, 6, 7 ^{3}H progesterone (specific activity 100ci/mol). These labelled steroids were obtained from New England, Nuclear (Southampton, U.K.). The overall recovery for estradiol 17_B and progesterone was 98.2 ± 5.1%. The RIA for estradiol 17_B and progesterone were performed using highly specific antisera. Estradiol was determined with an antiserum to 17_B-estradiol-6-(0-Carboxymethyl) oxime bovine serum albumin (Miles Laboratories, Slough, U.K.). Progesterone was measured with the use of antiserum to progesterone — 11α Hemisuccinyl-bovine serum albumin (Miles Laboratories, Slough). Coated immunoassay was performed. The sensitivity of the standard curve was 7 pg for estradiol 17_B and 5 pg for progesterone, applying 95% confidence limits.

The assay sensitivity was 40 pg/ml for estradiol 17_B and 0.5 ng/ml for plasma dilution 1:100 for progesterone. The intra-assay coefficient of variability (c.v.) was 8.6% for estradiol 17_B and 6.9% for progesterone. The inter-assay c.v. was 10.8% for estradiol 17_B and 9.4% for progesterone.

Blanks in all assays were replicated and below the sensitivity of the assay. The concentration of estradiol was expressed in picogram per millimetre; concentration of progesterone was expressed in ng/mm.

Statistical analysis

The mean s.e.m. were compared using the paired *t*-test.

Results

The mean number of corpora lutea on the operated side was 4.03 ± 0.32 while on the contralateral side (Control 1) was 6.466 ± 0.43. This difference was statistically significant ($P < 0.001$).

The mean progesterone from the operated side was 206.103 ± 13.296 while from the contralateral side it was 281.769 ± 13.58. This difference is also statistically significant ($P < 0.001$). The mean estradiol 17_B on the operated side was 128.57 ± 14.88 while on the contralateral side it was 159 ± 17.43. This difference is statistically significant ($P < .001$).

In eight unoperated animals (Control 2), the mean number of corpora lutea on the left side was 5.5 ± 0.78 compared to 5.8 ± 0.46 on the right side. The mean progesterone was 276.0 ± 26.8 on the left side compared to 255.77 ± 25.67 on the right side. The mean estradiol 17_B was 137.5 ± 30.44 on the left side and 130.0 ± 22.96 on the right side. These differences between left and right sides are not statistically significant. When data from right and left sides were pooled, they provided sixteen observations with a mean number of corpora lutea of 5.69 ± 0.44, mean progesterone of 265.89 ± 18.12, mean estradiol 17_B 133.75 ± 18.45. When this pooled data (Control II) was then compared to data from the operated side, the mean number of corpora lutea, mean progesterone and mean estradiol were lower on the

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operated side. Only the differences in the mean number of corpora lutea and mean progesterone were statistically significant ($P < 0.001$); ($0.02 > P < 0.01$) respectively. There was no correlation between the number of ovulations and estradiol or progesterone concentration.

Discussion

There is evidence for a close neuro-vascular relationship between the oviduct and its ipsilateral ovary (Mitchell, 1938). It therefore seems that damage to the fallopian tube could affect ovarian function. Our study shows clearly that ovulatory function is suppressed after salpingostomy following mechanically produced hydrosalpinges. The finding of reduced number of ovulation sites on the operated side is in agreement with previous animal studies (Koninckx *et al.*, 1980; McComb, Moon & Gomel, 1983; Beyth *et al.*, 1981).

In the present study, the contralateral ovary (Control 1) had more corpora lutea and the ovarian vein progesterone concentration was higher than in intact unoperated animals (Control 2). This may be due to a compensatory response. This may be one criticism of measurement of peripheral levels of hormones for comparison. Measurement of ovarian venous steroid concentration is a more direct assessment of ovarian function than is measurement of peripheral levels. It can be argued that only a gross abnormality of luteal function would be revealed by a single estimate of plasma progesterone in peripheral blood.

The validity of the measurements of estradiol and progesterone in this study is not in question as all samples were assayed against the same standard and the intraassay and interassay variability were 8.6%, 10.8% for estradiol 17_B and 6.9%, 9.4% for progesterone respectively. Mikhail, Noall and Allen (1961), measured progesterone levels in rabbit ovarian vein blood throughout pregnancy. They found that the concentration of progesterone rose gradually to reach a peak at day 14 post-coitum (p.c.). The concentration of progesterone they recorded on day 10 p.c. is higher than the values recorded in this study. This could be because RIA was not employed in their study; hence their assays lacked both specificity and sensitivity. The values of progesterone measured in this study are in agreement with those of Janson, Damber and Axen (1981), in pseudo-pregnant rabbits.

Day 10 p.c. was chosen for blood sampling in this study because it is a convenient time to count both the corpora lutea and the implantation sites. Implantation occurs in the rabbit on day 8 p.c. (Allen, Brambell & Mills, 1947). There was no correlation between the number of corpora lutea or implantations and the steroid output of an individual ovary. This finding is in agreement with that of Shaikh and Harper (1972). There were no significant variations between right and left ovaries (Control 2) in either the number of ovulations or steroid output. These findings contradict those of Shaikh and Harper (1972) who showed marked variations in secretion rates between ovaries within individual rabbits. In this respect, the ligation of the ovarian artery performed when cannulating the vein may have produced artefacts in their study. The variations they reported in their study may have resulted from prolonged occlusion of ovarian blood supply. This argument is supported by the study of Moor (1973) who demonstrated that the steroidogenic capacity of sheep ovarian follicles cultured *in vitro* was adversely affected by decreased oxygen tension.

The mechanism for suppressed ovarian function after surgical modification or disease of the Fallopian tube is not understood. Interference with the neurovascular connecting system between ovary and fallopian tube has been suggested (Koninckx *et al.*, 1980). The mammalian ovary receives adrenergic (sympathetic) and cholinergic (parasympathetic) nerves. The density of innervation varies considerably with species; it is very dense in guinea pigs, sparse in rabbits and moderate in humans (Jacobowitz & Wallach, 1967). Regardless of species and density of innervation, the distribution of nerve fibres in the ovary is similar in various mammalian species studied. Fluorescence microscopy has shown that these nerves are related to the vascular adrenergic nerves. The ovary also contains adrenergic nerves unrelated to the vessels (Rosengren & Sjoberg, 1968).

Unsickler (1974) showed that nerve fibres in the ovaries do not enter the granulosa layer on the follicles or corpora lutea. Nor was there evidence of a difference between innervation in non-pregnant and pregnant rats. Lawrence and

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Burden (1976), have shown that the number and intensity of interstitial fluorescent adrenergic nerves in the rat increases as pregnancy progresses. Measurements of neurotransmitters in the ovary showed there is a highly significant increase in neurotransmitter in the ovary on days 14 and 18 compared to day 4. Based on Unsickler's study, a direct effect of adrenergic nerves on steroidognesis is unlikely. Yet a direct effect of adrenergic nerves on the physical process of ovulation is likely (Walles *et al.*, 1975). It has been shown that an intra-aortic injection of Dibenzyline (an alpha-receptor site blocker), 7 and 10 h following an ovulatory dose of HCG (or 1–4 h before ovulation), decreased the number of ovulatory follicles (Virutamasen, Hickok & Wallach, 1971). It is therefore possible that follicular rupture, ovum maturation and ovarian steroid production can proceed independently from one another (Kitai *et al.*, 1983).

Apart from damage to neural connections, it may be that ampullary disease or surgery affects ovarian blood flow. We know that blood flow to the corpus luteum plays an important role in regulation of luteal function. Niswender *et al.* (1976), have demonstrated that, in sheep, blood flow to the ovaries containing a corpus luteum undergoes cyclic changes. The arteries supplying the ovary dilate during the luteal phase. Consequently blood flow to the ovary increases four- to six-fold. This increase in blood flow correlated with a rise in systematic plasma progesterone. Recently Janson *et al.* (1981) showed that ovarian progesterone secretion, assessed in ovarian venous blood in pseudo-pregnant rabbits positively correlated to the blood flow per unit weight of the luteal tissue. They concluded that a high rate of luteal blood flow may be necessary for optimal steroid production by the corpus luteum. Since there is a correlation between blood flow and steroidogenesis, vasomotor control by vascular adrenergic nerves may affect subsequent steroidogenesis.

Prostaglandins may play an important intermediary role. Davis, Horton and Withrington (1967), showed that sympathetic stimulation caused an increase in release of endogenous prostaglandin E_2 into splenic venous blood. This relationship between norepinephrine (NE) and prostaglandin (PG) release was explored further and substantiated by Stjarne (1973a and b). From his experiments, he hypothesized that NE has stimulatory effect on the release of PGE_2. PGE_2 may control the release of NE by negative feedback. Prostaglandin has also been shown to play a role in regulating the ovarian microcirculation. Novy and Cook (1973) showed that PGE_2 constricts the afferent blood vessels of the corpus luteum or the small arterovenous shunts in the ovarian circulation.

In a recent study by McComb, Moon and Gomel (1983), it was shown that the tissue concentration of prostaglandin E and F was significantly increased in the Fimbria of rabbit oviducts at the time of ovulation. Since prostaglandins play a role in ovulation, they suggested that removal of the fimbria, such as occurs after cases of salpingostomy, may be responsible for suppression of ovarian function.

Conclusion

Mechanically-produced hydrosalpinx and its surgical correction salpingostomy suppress ovarian function. This is probably due to interference with the neuro-vascular connection between the fallopian tube and the ovary. The mechanism by which such interference produces ovarian function suppression must be complex and is not understood.

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