Tubal and uterine secretions; the possibilities for contraceptive attack

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Introduction

In this article I shall be discussing our state of knowledge with respect to the composition and function of tubal and uterine secretions with particular reference to the possibilities for contraceptive attack. Before considering the secretions themselves it is important to establish the type of contraceptive we are hoping to develop as a result of these studies. One possible approach might be to search for specific proteins or glycoproteins in the genital tract secretions which are essential for the continuation of early embryonic development and which might, therefore, be used as targets for the immunological suppression of fertility. In both the tubal (Moghissi, 1970) and uterine (Roberts, Parker & Henderson, 1976; Maathuis & Aitken, 1978a, b) secretions of the human genital tract biochemical evidence exists for the presence of non-serum, possibly unique, proteins (Pl. 1, Fig. 1). The specificity of these proteins has not yet been established immunologically, however, and we may do well to remember that the protein “uteroglobin” (Krishnan & Daniel, 1967; Beier, 1968) was thought to be a specific component of rabbit uterine secretions for a decade until it was also discovered in the lungs, tubal secretions and seminal plasma of this species (Noske & Feigelson, 1976). Our uncertainty concerning the specificity of uterine and tubal proteins and the attendant hazards of autoimmune disease (Tung, 1976) should perhaps discourage us from this approach. An alternative route towards the immunological suppression of fertility might be to search for specific embryonic factors which are responsible for regulating the synthesis or release of critical components in the oviductal or uterine secretions. The advantage of this approach is that the target antigen is of embryonic origin and would, therefore, only be exposed to the maternal immune system for a limited period of time. The specificity of the embryonic factors involved would still have to be demonstrated however. Alternatively we might try to regulate the synthesis or release of critical secretory components by pharmacological means, using, for example, anti-oestrogens or anti-progestagens. Common to both the immunological and pharmacological approaches is the need to identify processes in early development in which tubal or uterine secretions play an important part. In the following sections I shall consider the possible roles played by these secretions and assemble the evidence for an embryonic or pharmacological influence on their composition.

The oviduct

Since the oviduct is the site of fertilization and subsequent cleavage of the embryo, oviductal secretions might play a role in such events as sperm capacitation, sperm binding, penetration of the zona pellucida, corona cell dispersal, the regulation of embryonic metabolism and the immunological protection of the zygote.

Capacitation of human spermatozoa can be readily achieved *in vitro* using a defined medium (such as M199) and a simple supplement of human serum albumin (3 mg/ml). Human spermatozoa incubated in this medium for 5 h become fully capacitated and acquire the ability to penetrate the zona-free oocytes of mice or hamsters and form pronuclei (Pl. 1, Fig. 2: R. J.

Aitken, D. Mortimer, D. W. Richardson & A. Templeton, unpublished observations; Hanada & Chang, 1972; Yanagimachi, 1972; Yanagimachi, Yanagimachi & Rogers, 1976). Since serum albumin is present in the tubal (Moghissi, 1970) and uterine (Maathuis & Aitken, 1978a, b) secretions of women at all stages of the cycle, there may be little prospect of interfering with capacitation for contraceptive purposes. Some evidence does exist, however, suggesting that sperm capacitation *in vivo* also involves the removal of a decapacitation factor acquired in the seminal plasma (Chang, 1957). Although this factor may be removed mechanically when spermatozoa are washed before fertilization *in vitro* the removal of the decapacitation factor *in vivo* may depend upon specific enzymes, possibly amylases, in the genital tract secretions. Another tubal factor which may be involved in the induction of sperm capacitation is calcium (Yanagimachi & Usui, 1974; Rogers & Yanagimachi, 1976; Reyes, Goicoechea & Rosado, 1978). The concentration of calcium in the tubal fluids of the rabbit (Holmdahl & Mastroianni, 1965) and rhesus monkey (Mastroianni, Urzua & Stambaugh, 1973) increases following ovulation and the presence of calcium, together with a divalent ionophore, A23187, accelerates the capacitation of rabbit spermatozoa *in vitro* (Reyes *et al.*, 1978). The removal of the decapacitation factor and the post-ovulatory influx of Ca^{2+} ions are both specific events which might serve as foci for the regulation of fertility.

The maintenance of sperm viability in the oviducts may also depend upon factors present in the tubal secretions, e.g. glucose (Bishop, 1969), lactate, pyruvate (Iritani, Gomes & Van-Demark, 1969a, b; Carlson, Black & Howe, 1970; Stone & Hamner, 1975), amylase (Hochberg, 1974), lactic acid dehydrogenase (Gibson & Masters, 1970; Lippes & Enders, 1972), glycerophosphoryl choline diesterase (Iritani *et al.*, 1969a) and an unidentified non-dialysable, heat-labile factor found in the genital tract of the ewe and rabbit (Iritani *et al.*, 1969b). The first stage of fertilization, the binding of spermatozoa to receptors in the zona pellucida, appears to be independent of any tubal factor because it will take place in a defined medium such as M199 supplemented with bovine serum albumin (Gwatkin, 1977; R. J. Aitken & D. W. Richardson, unpublished observations). However, the subsequent penetration of the zona pellucida by the spermatozoa does appear to be influenced by the oviductal secretions. Sperm penetration is preceded by the acrosome reaction which exposes a trypsin-like enzyme (acrosin), on the inner acrosomal membrane (Stambaugh & Buckley, 1968, 1969; Polakoski, Zaneveld & Williams, 1972; Zaneveld, Polakoski & Williams, 1972; Yanagimachi & Teichman, 1972) and this enzyme is thought to be responsible for digesting a path through the zona pellucida in advance of the penetrating spermatozoon. The importance of acrosin in the penetration process is indicated by the fact that fertilization *in vitro* (Stambaugh, Brackett & Mastroianni, 1969) and *in vivo* (Zaneveld, Robertson, Kessler & Williams, 1971) can be blocked by the presence of exogenous trypsin inhibitors. Endogenous protease inhibitors have been detected in the tubal secretions of women (Moghissi, 1970; Hirschhauser, Kionke & Daume,

EXPLANATION OF PLATE 1

Fig. 1. Disc gel electrophoresis on columns of 7% acrylamide, pH 8.9, of uterine flushings (UF) collected during the early secretory stage of the human cycle and the paired plasma sample. Non-serum proteins are indicated by arrows. Note the particularly dense band with *R_a* value of 0.41. (From Maathuis & Aitken, 1978a.)

Fig. 2. Murine oocyte containing 11 pronuclei derived from human spermatozoa capacitated in a defined medium (M199: Flow Laboratories) supplemented with 3 mg human serum albumin/ml.

Fig. 3. Polyacrylamide gel electrophoresis of mouse uterine flushings during Days 4–10 of pseudopregnancy. A = albumin, T = transferrin. Note the prominent high molecular weight proteins (arrowed) which appear in the uterine fluids on Days 8–10, as the mice are returning to oestrus.

PLATE 1

1971), rhesus monkeys (Stambaugh, Seitz & Mastroianni, 1974) and rabbits (McLaughlin, 1974). In the rhesus monkey a total of 6 protease inhibitors were identified in the oviductal fluids and 5 of them inhibited rhesus monkey acrosin. Two of these inhibitors were derived from serum ($\alpha 2$ -macroglobulin and $\alpha 1$ -antitrypsin) while the remainder were thought to be of tubal origin. The major protease inhibitors present in the tubal secretions of the rabbit (McLaughlin, 1974) and women (Moghissi, 1970) appear to be of serum origin. The purpose of these inhibitors is presumably to inhibit fertilization at all stages of the cycle apart from the immediate post-ovulatory phase during which their concentration declines significantly (Stambaugh *et al.*, 1974; McLaughlin, 1974). In this way the risk of fertilization involving ageing ova, with the attendant hazards of genetically abnormal offspring, is kept to a minimum. In this respect it is of interest that there exists in the human population a genetic variant, designated P1Z (Kueppers, 1973; Cooper, 1978), which is associated with a deficiency in the serum levels of $\alpha 1$ -antitrypsin, the major protease inhibitor in human tubal fluids. Carriers of the P1Z variant suffer from diseases of the lung and liver and, more importantly in the present context, give birth to a high percentage of chromosomally abnormal offspring (Fineman, Kidd, Johnson & Breg, 1976). It might also be predicted that low levels of $\alpha 1$ -antitrypsin in the tubal fluids would be associated with an increase in fertilization rate. If this were shown to be the case, it would explain how such a deleterious genetic variant has become established in the population (Kueppers, 1972).

Another event associated with fertilization which appears to involve the participation of the tubal secretions concerns the loss of the corona radiata from around the oocyte (Mastroianni & Ehteshamzadeh, 1964). The tubal factor responsible for dispersing the corona cells of the rabbit was shown to be dialysable and heat-stable and was subsequently identified as bicarbonate ion (Zamboni, Hongsanand & Mastroianni, 1965; Stambaugh, Noriega & Mastroianni 1969). The bicarbonate ion content of rhesus monkey tubal fluids has been shown to increase following ovulation (Maas, Storey & Mastroianni, 1977) and it is possible that this change is instrumental in stimulating the dispersal of the corona radiata in this species also. The mechanism by which the bicarbonate ion content of the tubal fluids is controlled is not known. Even so, it seems unlikely that the inhibition of corona cell removal would impede the progress of pregnancy.

There is some evidence to suggest that the next stage of embryonic development, cleavage, is associated with a change in the nature of the tubal secretions. In the rabbit, for example, the cleavage of fertilized oocytes appears to depend upon the removal of an oestrogen-induced inhibitor of embryonic development from the ampullary tubal fluids (Kille & Hamner, 1973; Stone & Hamner, 1975; Stone, Richardson, Hamner & Oliphant, 1977). This inhibitor of embryonic development has been identified in the oviductal fluids of oestrous rabbits and is known to be dialysable. A similar inhibitor appears to be present in the oestrogen-dominated tubal fluids of the mouse (Cline, Randall & Oliphant, 1977). The precise physiological significance of these inhibitors is not yet clear although it has been suggested that they serve to slow down "some initial events immediately after fertilization, to allow a proper sequence of development" (Cline *et al.*, 1977). Until definitive evidence for the presence of similar factors in primates has been obtained, their contraceptive potential will remain uncertain.

Another possible function of the oviductal secretions concerns the immunological protection of the zygote from the ravages of the maternal immune system. Using the rosette inhibition test as a criterion for immunosuppressant activity, Morton, Hegg & Clunie (1974) claim to have demonstrated the appearance of an immunosuppressant factor in the blood serum of mice within 6 h of copulation. Since it is improbable that significant quantities of this factor could be produced by the eggs themselves it is possible that the immunosuppressive substance(s) is synthesized by the oviduct in response to a stimulus delivered by the pronuclear-stage oocytes.

There is, therefore, some evidence to suggest that the tubal secretions play a part in the control of fertilization and embryonic development. We must now ask whether the synthesis or release of any of these components can be modulated by embryonic or pharmacological means. An influence of the developing embryo on the metabolic activity of the oviduct has been demon-

strated in the rabbit for such features as D,L-[1-¹⁴C]valine incorporation (Roy, Gupta, Roy, Karkun & Kar, 1972) and the ampullary concentration of lactic acid and acid phosphatase (Gupta, Karkun & Kar, 1970). Similarly the presence of an embryo has been found to influence [³H]thymidine incorporation by the mouse oviduct (Freese, Orman & Paulos, 1973). From a contraceptive point of view elucidation of the nature and extent of these interactions between the embryo and oviduct would seem to be a profitable avenue for research. With respect to the pharmacological control of oviductal secretory activity oestrogen appears to be responsible for such changes as the disappearance of protease-inhibitor activity (McLaughlin, 1974), the presence of inhibitors of embryonic growth (Cline *et al.*, 1977; Stone & Hamner, 1977) and the appearance of such essential components as calcium, glucose, lactate and pyruvate (Holmdahl & Mastroianni, 1965). It is, however, difficult to envisage any regimen by which hormones (or anti-hormones) could be administered to interfere with normal tubal function without disrupting ovulation.

The uterus

Oestrous uterine secretions

In all species so far investigated, including man, ovulation is associated with the production of a voluminous, protein-rich uterine secretion of low viscosity (Lutwak-Mann, Bourns & Bennett, 1960; Fahning, 1965; Aitken, 1970, 1977a; Clemetson, Kim, DeJesus, Mallikarjunswara & Wilds, 1973; Meglioli, Kalvoda & Desaulles, 1973). The physical function of these uterine fluids is to dilate the uterine lumen and thereby create a passage through which the spermatozoa can progress towards the utero-tubal junction. While they are suspended in this fluid the spermatozoa undergo the initial stages of capacitation (Yanagimachi & Usui, 1974). Although the initiation of capacitation does not seem to be a very exacting function for these fluids, they have a surprisingly complex composition incorporating a large number of non-serum, possibly unique, proteins (Aitken, 1977b, c; Surani, 1977; Pl. 1, Fig. 3). It would be fascinating, and perhaps of practical importance, to know the precise functions of these components.

Progestational uterine secretions

During early pregnancy the uterus produces a second type of secretion which serves to induce and maintain blastocyst activity over the implantation period. The role of these secretions in the stimulation of embryonic growth became evident as a result of studies on species exhibiting the facultative and obligate forms of delayed implantation such as the roe deer (Aitken, 1974a, b, c), wallaby (Renfree, 1973), fur seal (Daniel, 1971), mouse (Aitken, 1977b, c) and rat (Surani, 1975). In all these species the reactivation of the blastocysts at the end of diapause is temporally associated with an increase in uterine secretory activity. Subsequent research has concentrated on determining the secretory factors responsible for stimulating the blastocysts. The first species to be studied in depth was the rabbit in which implantation was found to be associated with the appearance in the uterine fluids of a major protein termed uteroglobin (Beier, 1968) or, in view of its predicted embryotrophic properties, blastokinin (Krishnan & Daniel, 1967; Daniel, 1968). A search for similar proteins in other species then followed which, with the possible exception of the fur seal (Daniel, 1972a) and man (Daniel, 1973), was unsuccessful. It is now apparent that in most species the progestational uterine secretions consist of a complex mixture of proteins (Surani, 1977; Aitken, 1977b, c; Pratt, 1977), many of which are thought to have a transport function. Uteroglobin, for example, is now thought to be a progesterone-binding protein (Beato, 1977). In the mouse (Aitken, 1977a), wallaby (Renfree, 1973), rabbit (Daniel, 1972b) and pig (Wyatt, Heap & Perry, 1976), the evidence suggests that the implanting or elongating blastocyst may have a local stimulatory effect upon the secretory activity of the endometrium. Such an interaction may be particularly important in women in

whom the quantity of protein released into the uterine lumen during the luteal phase of the non-pregnant cycle is minimal (Maathuis & Aitken, 1978a). It is therefore possible that the development of full secretory activity in the human uterus requires the presence of a blastocyst. With this possibility in mind we might reflect upon the importance of elucidating the nature of the interactions between blastocyst and endometrium in appropriate animal models.

Hormonally, the control of uterine secretory activity in species not exhibiting delayed implantation, such as the rabbit (Arthur & Daniel, 1972) or pig (Knight, Bazer & Wallace, 1973), is thought to be mediated by progesterone. For implantation to take place it is important that these progesterone-dependent secretions are released into the uterine lumen shortly after blastocyst formation. If exogenous steroids are used to delay (Beier, Kühnel & Petry, 1971; Adams, 1973, 1976) or accelerate (McCarthy, Foote & Maurer, 1977) release of these secretions in a species such as the rabbit, implantation is prevented. Whether a similar approach could be used to inhibit implantation in women is still open to question. However, the absence of a discrete secretory peak in the mid-luteal phase of the human (Maathuis & Aitken, 1978a, b) and baboon (Peplow, Breed, Jones & Eckstein, 1973) cycle is discouraging.

A major secretory product of the luteal-phase human endometrium is prostaglandin (PG) F-2 α (Pickles, Hall, Best & Smith, 1965; Downie, Poyser & Wunderlich, 1974; Singh, Baccarini & Zuspan, 1975; Jordon & Pokoly, 1977; Maathuis & Kelly, 1978). This compound does not appear to be involved in the control of luteal function in women (Beling, Marcus, Markham, 1970; LeMaire & Shapiro, 1973; Aitken & Harper, 1977) as it is in many other species (Aitken & Harper, 1977) but may be instrumental in initiating the events leading to menstruation. In contrast to the non-pregnant cycle, the endometrial concentration of PGF-2 α is uniformly low during early pregnancy in man (Maathuis & Kelly, 1978). This observation may be explained in one of two ways: either the decidualized endometrium is incapable of PGF-2 α synthesis or the implanting conceptus produces a factor(s) which suppresses local PGF-2 α anabolism. The latter possibility is the more likely because one of the patients examined by Maathuis & Kelly (1978) was only 17 days pregnant and despite a normal late luteal-phase endometrial biopsy the PGF-2 α levels in the tissue sample were minimal. Isolation of the embryonic factors responsible for the suppression of endometrial PGF-2 α synthesis in women would seem to be a particularly promising area for research which might lead to the identification of an ideal target for contraceptive intervention.

Conclusion

The tubal and uterine secretions appear to offer several potential opportunities for contraceptive attack. The realization of this potential will, however, depend upon a shift of research emphasis away from the secretions *per se* and towards their interactions with the embryo.

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