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DIRECTIONAL FLOW OF FALLOPIAN TUBE
SECRETIONS IN THE ROMNEY EWE

by

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CONTENTS OF THESIS

Summary

Chapters	Page
I. The Fallopian Tube and its Function in the Transport of Spermatozoa and Ova.	1
II. Directional Flow of Fallopian Tube Secretions in the Romney Ewe.	16
III. The Effect of Oestrogen Therapy on the Directional Flow of Tubal Secretions in the Ovariectomised Ewe.	32
IV. The Effect of Oestrogen-progesterone Sequential Therapy on the Directional Flow of Tubal Secretions	58
V. The Directional Flow of Tubal Secretions in the Entire Ewe During Silent Oestrus.	71
VI. General Discussion	79
VII. Bibliography	88

SUMMARY

The anatomy of the Fallopian tube and the function of its components, especially muscle and cilia, were discussed in reference to their participation in sperm and ovum transport. The possibility of instantaneous sperm transport in the female reproductive tract and the extent to which the inherent motility of spermatozoa is required were noted as topics of long standing controversy. In contrast, evidence in the literature on the rate of ovum transport was reasonably consistent. While considerable evidence was available to indicate the relative contribution of muscular and ciliary activity only limited data were found on the role of tubal secretions. That the direction of fluid flow was controlled, and possibly regulated by ovarian hormones, was shown by few authors.

The continuous collection of fluid flowing from both ends of the Fallopian tube of the ewe was therefore attempted by placing a cannula in the ovarian end of the ampulla and a further cannula passing through the wall of the corresponding uterine horn and opposed to the utero-tubal junction. By experimentation the technique of cannulation and collection of fluid was improved and a standard procedure adopted. Daily recordings of fluid were made with 40 entire ewes over a period ranging from 3 - 106 days after surgery during the breeding season. Observations were made for oestrous behaviour.

There was an increase in total output of tubal fluid, which commenced on the last day of the oestrous cycle and reached a maximum about day 2 after which a gradual decline occurred. A greater part of the fluid secreted flowed through the ampulla end. Flow through the utero-tubal junction into the uterus remained low for most of the cycle but markedly increased in all cycles 3.9 ± 0.1 days after the onset of oestrus. Thus maximum flow through the isthmus appeared to

coincide with the time during which ova enter the uterus.

To determine the hormonal control of fluid flow a series of experiments on ovariectomised ewes were undertaken. The principle ovarian hormones, oestrogen and progesterone, were given in single, serial, or sequential doses. A 'positive oestrous response' (Robinson & Moore, 1956b) to oestrogen therapy was indicated by the subsequent cornification of vaginal epithelial cells.

A decline in fluid secretion and fluid flow following ovariectomy was counteracted by the administration of oestradiol benzoate. Both ampullar and isthmie flow markedly increased (with single injection of 30, 90 or 500 μ g ODB) until it was commensurate with, or exceeded, that which occurred during oestrus in the entire ewe. But, the sequence of maximum ampullar flow and that of isthmie flow was reversed by oestrogen therapy, the difference being further accentuated by each increase in dose level. Furthermore, serial therapy (30 μ g ODB daily for 3 days) so extended the duration of isthmie flow that it offset a comparable increase in ampullar flow.

In an attempt to achieve the sequence of fluid flow as it occurred in the oestrous animal the normal hormonal balance was simulated by sequential progesterone-oestrogen therapy. A preparatory treatment with progesterone (10 mg daily for 8 days) depressed fluid secretion and in consequence the flow of fluid out both ends of the Fallopian tube. The response of fluid secretion and isthmie flow to subsequent oestrogen therapy was also reduced. This was reflected by a decrease in response of maximum fluid secretion and isthmie flow and also a reduction in the duration of response. The progesterone therapy, given on the third day after oestrogen, further decreased the duration of response. Thus an increase in isthmie flow, expected to occur with the succeeding progesterone therapy, did not eventuate.

The sequence of fluid flow was essentially the same as that occurring after oestrogen alone.

While the oestrogen-induced isthmic flow was not completely suppressed by preparatory treatment with progesterone it was reduced. Thus it had been surmised that progesterone preceding oestrogen therapy may be necessary to suppress isthmic flow during the oestrogen dominant pre-ovulatory period of the entire ewe.

Supporting evidence on this point was obtained from data on the direction of fluid flow during silent heat. The sequence of maximum ampullar flow and that of isthmic flow in the 'first oestrous' (silent heat) was similar to that which occurred in the oestrogen treated ovariectomised ewe. In contrast, the sequence of fluid flow in the subsequent oestrous cycle was reversed, being similar to that occurring in the oestrous ewe.

It was concluded that the direction of fluid flow was under hormonal control; fluid secretion and isthmic flow being promoted by oestrogen therapy but depressed by progesterone. In addition, progesterone emanating from the waning corpus luteum of a preceding ovulation was considered as playing a decisive role in suppressing isthmic flow during the pre-ovulatory period of the oestrous ewe. However, the hormonal balance necessary to enable the surge in isthmic flow on the third day after ovulation was not determined.

The significance of these results, and their limitations, were then discussed in terms of spermatozoan and ovum transport. It was concluded that tubal secretions have an integrative function in the transport mechanism. The fluid was considered as a medium through which the effect of ciliary and muscular activity could be transmitted. The movement of ova, spermatozoa and fluid was considered synonymous.

CHAPTER ONE

THE FALLOPIAN TUBE AND ITS FUNCTION IN THE TRANSPORT OF SPERMATOZOA AND OVA

CONTENTS OF CHAPTER

A.	INTRODUCTION	Page 1
B.	ANATOMY OF THE FALLOPIAN TUBE	
	1. Morphology	1
	2. Histology	2
C.	TRANSPORT OF SPERMATOZOA IN THE FEMALE GENITAL TRACT	3
	1. Evidence for Passive Transport	
	(a) Time of Transit	4
	(b) Passage of Inert Particles	5
	2. Mechanisms for Sperm Transport	5
	(a) Muscular contractions	6
	(b) Barriers to sperm progress	6
	(c) Anti-peristaltic contractions	7
	(d) Compartmental theory	8
D.	THE TRANSPORT OF OVA THROUGH THE FALLOPIAN TUBE	8
	1. Rate of Normal Transport	9
	2. Mechanisms for Ova Transport	9
	(a) Entry of ova into the Fallopian Tube	10
	(b) Transport through the Fallopian Tube	11
	(i) muscular contractions	11
	(ii) ciliary activity	12
	(iii) restrictive action of the isthmus	13
E.	PURPOSE OF INVESTIGATION	14

A. INTRODUCTION

Each normally implanted embryo is preceded by the successful transfer of spermatozoa and ova to the ampulla of the Fallopian tube, fertilisation, and subsequent delivery of fertilised ova to the uterus after a suitable period of time. Any malfunction in the mechanisms controlling these processes can seriously interfere with the ability of an individual, or species, to reproduce. It is therefore necessary to gain a closer understanding of the physiological mechanisms controlling these events.

The review presented in this chapter outlines present knowledge on the mechanics, and time relationships, of normal entry and passage of gametes through the Fallopian tube.

B. ANATOMY OF THE FALLOPIAN TUBE

1. Morphology

The Fallopian tubes are bilaterally placed, each being suspended by a mesenteric peritoneal fold, the mesosalpinx. The following regions of each Fallopian tube are easily recognised (Piliero, Jacobs & Wischnitzer, 1965):

1. The funnel shaped infundibulum containing a fimbriated opening, the abdominal ostium, projecting towards and in contiguity with the ovary.
2. An intermediate dilated portion, the ampulla.
3. The isthmus, a constricted and convoluted segment, providing continuity between its uterine orifice, the utero-tubal junction, and the ampulla.

The vascular system of the Fallopian tube has not been clearly defined. Arterial blood originates from anastomoses of the uterine and utero-ovarian arteries in the cow (Hansel & Asdell, 1951), ewe

(Sisson & Grossman, 1953), and appears to be similar in the sow (Oxenreider, McGlure & Day, 1965). The venous system follows a pattern similar to the arterial while lymph drainage occurs via the lumbar and inguinal nodes (Sisson & Grossman, 1953).

Sympathetic innervation links with the ovarian and hypogastric plexus. Recent evidence reported by Brundin (1964a) suggests ampulla innervation is cholinergic, while fluorescent staining techniques (Brundin & Wirsén, 1964b) indicated adrenergic nerve terminals in the isthmus of the rabbit.

2. Histology

The Fallopian tube is organised into 3 layers, a thin peripheral serosa, a muscularis, and the internal mucosa.

The muscularis consists of two poorly defined muscle layers, a circular and a longitudinal layer. The layer of circular muscle is slightly spiralling (Greenwald, 1961), and partly interlocked with longitudinal muscle (Bjork, 1959). The presence of longitudinal muscle in the ampulla of the doe (Black & Asdell, 1958) is doubted by some authors. Greenwald (1961) claimed it appeared a few centimetres before the uterus as two distinct bands which gradually widened to form a complete layer around the uterus. No contemporary reports are available for the distribution of the muscle layers in the Fallopian tube of the ewe.

The internal mucosa is lined by a single columnar epithelium. Sectioning of the ampulla reveals the mucosal lining thrown into a series of longitudinal folds which decrease in height and complexity as one moves into the isthmic region.

Three cell types: "Ciliated", "non-ciliated secretory" and "rod" cells, are present in the tubal epithelium of the guinea pig, rabbit, cat, dog, sheep, monkey and human Fallopian tubes (Frommel, 1886; Nicolas, 1890). The presence of similar and other cells in further

species was confirmed by a series of authors around the turn of the century (cited Hadek, 1955). Hadek confirmed previous suspicions that the rod cells were degenerate secretory cells, but did not support an early contention that secretory cells were transformed ciliated cells. This objection was later favourably supported by colchicine studies in the rabbit (Fredericsson, 1959a) and electron microscopy in the rabbit (Borell, Nilsson, Wersall & Westman, 1956) and human (Clyman, 1966). Secretory cells were found to be most abundant in the infundibulum and ampulla but comparatively few present in the isthmus of the sheep (Hadek, 1955; Restall, 1966c).

The epithelial folds of the rabbit, sheep and human ampulla are covered with an almost continuous layer of cilia, especially nearer the fimbria. They appear less numerous in the mid isthmus but are again predominant on the folds of the utero-tubal junction (Hadek, 1955; Bjork, 1959; Greenwald, 1961a; Harper, 1961b; Clyman, 1966). The earlier evidence of Espinasse (1935) suggesting cilia were absent from the isthmus of the rat and mouse was refuted by the more refined techniques of Alden (1942), Deane (1952) and Borell, Gustavson, Nilsson & Westman (1959).

The main components of the Fallopian tube which are directly implicated with the transport of the gametes have been outlined. The part played by each will be discussed subsequently.

C. TRANSPORT OF SPERMATOZOA IN THE FEMALE GENITAL TRACT

The relative contributions of sperm motility, and uterine and tubal contractions, towards sperm transport through the reproductive tract is still a controversial topic. Differences arose from the discrepancy between the almost instantaneous sperm transport in the female genital tract and the much slower progress of sperm in vitro. Reports indicate the mean speed of travel in vitro is less than

8 mm/min. (Phillips & Andrews, 1937; Tampion & Gibbons, 1962), although within species this may be increased if an opposing fluid current is created (Yamane & Ito, 1932). Even so, the rate of progress obtained does not account for the rapid transport which has been found to occur in many species. The occurrence of rapid transport, and the ascent of inert particles, has led many authors to propose a more passive role for spermatozoa. Contributions to the opposing views have been reviewed by Reynolds (1939), Dauzier (1958), Bishop (1961) and Hancock, (1962).

1. EVIDENCE FOR PASSIVE TRANSPORT

(a) Time of Transit

Hartman & Ball (1930) and Warren (1938) were among the first to demonstrate the presence of sperm in the Fallopian tube of the rat within 2 minutes of copulation. Subsequent estimates in other animals have placed the time at 15 minutes in the mouse (Lewis & Wright, 1935), 20 min. in the bitch (Evans, 1933), while estimates in humans range up to 3 hr (Chang & Pincus, 1951). Results with cattle and sheep vary considerably. With the cow, Van Demark & Moeller (1950, 1951) claim a few minutes but in contrast Brewster, May & Cole (1940) and Dauzier (1958) consider several hours more appropriate. In the ewe rapid ascent may (Phillips & Andrews, 1937; Schott & Phillips, 1941; Starke, 1949) or may not occur (Green & Winters, 1935; Kelly, 1937; Warbritton, McKenzie, Berliner & Andrews, 1937; Lopyrin & Loginova, 1939). The rabbit, on the other hand, appears to be an anomaly as rapid transport has not been reported to occur (Braden, 1953; Turnbull, 1966).

In his review Dauzier (1958) considered that inadequate precautions had been taken against possible sources of sperm contamination. For this reason he was skeptical of results showing rapid transport in the ewe and cow. This criticism can not be disregarded but the

position was perhaps clarified by Mattner & Braden (1963a) when they found the concentration of sperm in the ampulla of the ewe at 8 to 30 minutes was considerably reduced if the animals were mated when subjected to stress.

(b) Passage of Inert Particles

Additional evidence for a passive role of spermatozoa comes from the rapid transport to the ampulla of immotile sperm in the rat (Howe & Black, 1963), ewe (Mattner, 1963c), and cow (Van Demark & Moeller, 1951). Also the passage of radiopaque fluids (rabbit - Krehbiel & Carstens, 1939; Akester & Inkster, 1961; cow - Rowson, 1955), indian ink particles (rabbit - Parker, 1931), ^{131}I labelled microspheres (rabbit - Glover & Wood, 1964), and the ascent of carbon particles (ewe - Mattner, 1963a). In the sow and mare components of seminal plasma, ergothionine, fructose and citric acid, are often found in the uterine horns following mating (Mann, Polge & Rowson, 1955). Using a similar approach Gunn & Gould (1958) detected a zinc labelled component of prostatic fluid present in the Fallopian tube of the rat 1.5 hr after mating.

These observations have not passed unopposed. Noyes, Adams & Walton (1958), Bjork (1959) and Edgar & Asdell (1960) were unable to confirm the entry of radiopaque material into the uterus of the rabbit while Leonard & Pearlman (1939) failed to find non-motile sperm passing through the utero-tubal junction of the rat. However, each of these techniques involve mating, or inseminating, under conditions of possible stress and so preconditioning of the animals is probably a prerequisite for the occurrence of rapid transport.

2. MECHANISMS FOR SPERM TRANSPORT

If the inherent motility of the sperm is not obligatory for transport to the ampulla then it is necessary to seek other means to account for the displacement of sperm in the female genital tract.

Ciliary activity and muscular contractions of the uterus and Fallopian tube have been implicated (Westman, 1926; Parker, 1931).

(a) Muscular Contractions

Using direct observations Westman (1926) concluded that the muscular activity was peristaltic in nature. Subsequent work tended to refute this and considered the contractions to be propagated by a segmentation process (Maeda, 1933, Bjork, 1959) although intermittent peristaltic contractions may occur (Harper, 1961b). However, such peristaltic contractions that do occur are directed from the ampulla to the cervix (Reynold, 1939) and so barely able to induce sperm transport in the ovarian direction.

Segmental contractions encourage dispersal of uterine and ampulla contents as seen following the deposition of indian ink (Parker, 1931) or radiopaque materials (Bjork, 1959). The increase in uterine and tubal activity coinciding with oestrus presumably increases the turbulence of fluid within the genital tract thereby enabling more rapid dispersion of spermatozoa (Parker 1931).

(b) Barriers to Sperm Progress

Three possible barriers to sperm progress exist; the cervix, the utero-tubal junction, and to a lesser extent the narrow confines of the isthmus. In those animals in which sperm are deposited in the vagina during coitus, as occurs in the rabbit, ewe and human (Warbritton et al., 1937; Chang, 1951b; Braden, 1953; Hartman, 1957; Noyes, Adams & Walton, 1958), the mechanisms promoting the passage of sperm across the cervix are not definitely known. One theory, recently discussed by Hartman (1957), involves a transient negative pressure in the uterus which 'sucks' the sperm through the cervix. Millar (1952) in fact recorded a transient negative pressure of 0.7 lb per square inch in the uterus of the mare during coitus but the significance of this find is somewhat clouded if ejaculation normally occurs

directly into the uterus in this species (Braden & Austin, 1953b). The mass uptake of radiopaque fluid from the vagina into the uterus observed by Krehbiel & Carstens (1939) was considered due to vaginal contractions, but the conditions in which the rabbits were stimulated may have been abnormal (Akester & Inkster, 1961).

That the inherent motility of spermatozoa may be necessary for their transport across the cervix (Noyes et al., 1958) has been emphasised by Glover & Wood (1964) when they found only .002% to .04% of the copolymer microspheres deposited in the vagina of the rabbit eventually reached the uterus. This appears considerably less than the percentage of spermatozoa which reach the uterus (Braden, 1953), although a possible difference between strains of rabbits and degree of vulva stimulation cannot be discounted.

The resistance of the utero-tubal junction to sperm transport appears to vary between species. The speed of sperm transport through this region of the genital tract is generally rapid and in only the rat and rabbit (cited Braden, 1953) and possibly the sow (Rigby, 1966) is it considered an obstacle to sperm migration. The passage of radiopaque materials through the junction indicates a role for uterine contractions in the movement of fluids into the isthmus. Alden (1942b) noted that it was uterine fluid which partly filled the isthmus in the rat, while recently (Hawk, 1965) failed to find any sperm in flushings of the Fallopian tube in the ewe following the insertion of a plastic spiral in the corresponding uterine horn.

(c) Anti-peristaltic Contractions

Wintenberger - Torres (1961) found plastic spheres, comparable in size to natural ova, moved in an adovarian direction if placed at the base of the excised isthmus of the oestrus ewe. Whether the in vitro conditions and the nature of the spheres allow their argument for the existence of antiperistaltic contractions to be valid or extrapolated to the transport of spermatozoa is questionable. It

is worthy of note that Lim & Chao (1927), among other earlier authors, considered anti-peristaltic contractions of the isthmus as a possible mechanism for inducing sperm movement towards the ampulla.

(d) Compartmental Theory

Following his investigations in the rabbit, Parker (1931), proposed a 'compartmental' theory to account for sperm movement in the Fallopian tube. He contended that the epithelial folds of the internal mucosa form temporary compartments within which ciliary activity create aduterine and adovarian currents of fluid. The segmental contractions of the tubal musculature ensure a continuous dissolving and reforming of these compartments. Under such conditions fluid streams in one compartment can become part of, or continuous with, fluid streams in adjacent compartments.

Thus the dynamic state of compartmental fluid circulation would act as a media for the gradual dispersion of spermatozoa towards the infundibulum. The validity of this contention remains to be tested.

D. THE TRANSPORT OF OVA THROUGH THE FALLOPIAN
TUBE

The pattern of normal ova transport through the rabbit Fallopian tube has been described by Tourneux (1889), Parker (1930), Chang (1951c), Braden (1953), Greenwald (1959a, 1961a), Zimmerman (1959), Harper, Bennett, Boursnell & Rowson (1960), and Harper (1961a,b). It has also been described in the guinea pig (Squier, 1932), the sow (Corner, 1921; Anderson, 1927; Pomeroy, 1955; Perry & Rowlands, 1962; Oxenreider & Day, 1965b), the ewe (Kelly, 1937; Wintenberger, 1953) and the cow (Hamilton & Laing, 1946).

In their reviews on this topic, Anderson (1927), Chang & Pincus (1951a) and Blandau (1961) concluded that the time taken for ova to reach the uterus was remarkably constant between species. Apart from the sow (Oxenreider & Day, 1965b) dog and ferret (cited Hammond & Walton,

1934) the time taken, 3-4 days, appears to be independent of the size of the animal concerned or the length of the Fallopian tube.

1. RATE OF NORMAL TRANSPORT

Since few ova have been flushed from the ovarian half of the ampulla in the rabbit (Black & Asdell, 1959, Greenwald, 1961a) the ewe (Kelly, 1937; Wintenberger, 1953) and sow (Anderson, 1927) these authors concluded that ova transport through the ampulla of these species was rapid. Wintenberger (1953) noted ova had already passed through the upper half of the ampulla by 2.5 hr in the ewe. In the rabbit, where larger numbers of ova released allow more precise estimates, Zimmerman (1959), put the time of ampulla passage at 30-45 minutes. Recently, Harper (1961a), reported that rabbit ova in cummulus, when placed in the ovarian end of the ampulla, were transported to the ampulla-isthmus junction in 5 minutes (range 3.5 - 6 minutes).

It is therefore evident that ova spend the greater part of the 3 days following ovulation traversing the isthmus. The infrequent location of ova in the uterine half of the isthmus (Andersen, 1927; Kelly, 1937; Greenwald, 1959a) indicates ova may also be rapidly transported through this section of the Fallopian tube. However, considerable dissention exists between the reports on the site at which ova are delayed in the isthmus. One faction (Wintenberger, 1953, 1961; Greenwald, 1961a;) consider ova to be retained at the ampulla-isthmic junction for a prolonged period while others (Black & Asdell, 1958, 1959; Noyes, 1959; Edgar & Asdell, 1960) propose that the site of retention is immediately prior to the utero-tubal junction.

2. MECHANISMS FOR OVA TRANSPORT

It will be apparent that two distinct phases of ova transport occur. These two phases: entry of ova into the Fallopian tube, and transport through the ampulla and isthmus, have different underlying mechanisms inducing egg movement.

(a) Entry of ova into the Fallopian tube

The fimbria is known to partially surround the ovary for a brief period during ovulation in the guinea pig, rabbit, sheep, monkey and human (cited Westman, 1952; Doyle, 1956). In these species close approximation of the fimbria and ovary is ensured by an increase in motility and turgidity of the fimbria, and the rhythmic contractions of the musculature of the peritoneal fold and ovarian ligaments (Westman, 1926, 1952; Reynolds, 1939).

The process of ovulation has been directly observed and described in detail in the rabbit (Walton & Hammond, 1928; Hill, Allen & Kramer, 1935; Harper, 1963) and in the ewe (McKenzie & Terrill, 1935, 1937). Rupture of the follicle was explosive in nature and subsequent expulsion of follicular contents took only 30-60 seconds (Hill et al., 1935). However ovulation may not be explosive in all cases (McKenzie & Terrill, 1937) nor the ovum expelled until some minutes after rupture of the follicle (Harper, 1963). The mechanism whereby ova are transferred from the ovarian surface to the ampulla remains to be elucidated. One of the earlier theories involve the rhythmic relaxation and contraction of the ampulla creating a negative pressure and thereby drawing ova into the tube (Westman, 1926). Investigating this possibility Clewe & Mastroianni (1958) and Hafez (1961) placed a ligature around the ampulla immediately below the fimbria of the rabbit. No differences were apparent when a comparison was made of the number of ova reaching the fimbrial portion of the ligated and non-ligated tubes, thus discounting suction as a major factor in the 'pick-up' of ova.

The existence of numerous cilia (Borell et al., 1956) and that rabbit ova in cumulus move in a smooth continuous motion in this region (Harper, 1961b; Wintenberger-Torres, 1961) lends support to the more recent concept (Alden, 1942a,b; Blandau, 1958) which implicates ciliary activity as playing a major part.

(b) Transport through the Fallopian tube

Early discussions on the mechanism of ova transport through the Fallopian tube (Lode, 1893; Sobotta, 1914; Mikuliez - Radecki, 1925; Westman, 1926; and Kuo & Lim, 1928) considered muscular contractions and ciliary activity to be the main factors involved. The relative significance of these two factors remained undecided in subsequent reviews (Parker, 1931; Alden, 1942; Pincus & Chang, 1951; Blandau, 1961). More recently evidence has implicated a physiological ampulla-isthmus sphincter (Greenwald, 1961a), a restriction of the lower isthmus (Black & Davis, 1962) and the utero-tubal junction (Black & Asdell, 1959) may also be involved.

(i) muscular contractions:

Aduterine peristaltic contractions were observed in the human (Rubin & Bendick, 1926) and rabbit (Westman, 1926). In the following year Dryoff (1927) noted clear 'spindel' formations following the injection of a contrast medium into the Fallopian tubes of women. During the succeeding years, after considerable confusion as to the direction and type of contractions, it became evident that the direction of fluid movement depended on where the contrast medium was placed. Thus Nahmacher (1929) reported peristaltic contractions towards the ovaries in the human when contrast medium was placed in the uterus, but in the opposite direction if the medium was placed in the abdominal opening of the ampulla. Conveyance of a contrast medium has been used to confirm the occurrence of tubal contractions in both directions in many experiments since that time (Bjork 1959).

In priori to Westman's peristaltic contractions Parker (1931) and Maeda (1933) introduced the term 'segmentation' to describe the contractions which they observed in the Fallopian tube of the rabbit. Segmentation is presumably synonymous with Alden's (1942a) pendular contractions which caused violent back and forward movements of tubal

contents in the ampulla of the rat. Similar 'pendulum' or 'segmental' movements in the Fallopian tube of the mouse (Whitney & Burdick, 1936; Burdick, Whitney & Emerson, 1942) the rabbit (Black & Asdell, 1958; Bjork, 1959; Harper, 1961b; Inkster, 1964) the pig (Seckinger, 1923) and sheep (Wintenberger-Torres, 1961) have been reported.

In contrast, Wintenberger-Torres (1961) have reported antiperistaltic activity in the in vitro isthmus of the ewe. These singularly directional contractions were considered to aid sperm transport (refer page 7) and also prevent ova from entering the isthmus until the third day following ovulation. Supporting evidence under in vivo conditions may not be easily obtained.

(ii) ciliary activity:

Some authors, particularly Grosser (1918), maintained that tubal cilia were the only means of transporting ova through the Fallopian tube. Parker (1931), Anderes (1941), Borell, Nilsson & Westman (1957) and others, have discussed the relative importance of cilia in ova movement. Employing high speed cinematography Borell et al., (1957) found cilia beat toward the uterus at a rate of 1500 beats/min. during oestrus in the rabbit, then increased to 1600 at 24 hr, and 1800 beats/min. at 48 hr after copulation. Since maximum ciliary activity coincided with the time during which ova enter the isthmus the authors concluded that ciliary activity was significantly involved in the movement of ova through the isthmus. Previously Parker (1931) pointed out that cilia would only be effective in moving the ovum towards the uterus if the epithelial folds within the tube maintained a steady pressure thus ensuring reasonable ciliary contact. Even so, cilia have been observed to rotate a cluster of ova in the ampulla of the mouse (Burdick et al., 1942). Reversal and reanastomoses of a small section of the tube in the rabbit prevented pregnancy but reanastomoses without reversal did not (Kuo & Lim, 1928). Kuo & Lim extrapolated these

results to indicate that reversal of ciliary beats disrupted the transport of ova, although disruption of sperm transport, or muscle activity, cannot be ignored as possibilities. It is perhaps significant that ovum movement is most rapid in the ovarian and uterine quarters of the Fallopian tube where cilia concentrations are greatest.

(iii) restrictive action of the isthmus:

The exact location and cause of the physiological sphincter in the isthmus remains uncertain. While anatomic sphincters at the utero-tubal junction have been demonstrated in many species (Anderson, 1927) none have been demonstrated at the ampullar-isthmic junction (Greenwald, 1961a).

Muscular activity of the Fallopian tube increases as the time of ovulation approaches but quickly declines following ovulation (Westman, 1926). Intraluminal pressure changes in the isthmus and ampulla of the rabbit were recorded by Greenwald (1963b). Contractions of the ampulla at oestrus, and the two succeeding days, were rapid but relatively weak and highly irregular in amplitude. The isthmic contractions occurred at the same rate as those of the ampulla but were more uniform and of greater amplitude. Over the next three days however, isthmic contractions became progressively less frequent with powerful prolonged contractions being interspersed with others of lower amplitude. This decrease in the frequency of isthmic contractions coincided with the isthmic passage of ova. Greenwald concluded that the initial intense muscle activity in the isthmus presumably created a restrictive action on ovum entry into this region. Under in vitro conditions Brunden (1964a) induced marked increases in intraluminal pressure in the ampulla and isthmus using doses of acetylcholine and noradrenaline respectively. The failure of increases in ampulla pressure reaching the uterine strain gauge transducer, and the converse, indicated a functional isthmic occlusion in the rabbit. Indeed, this

was the case even when the two records were taken from points 1.5 cm apart and separated only by the ampulla-isthmic junction (Brunden, 1964c).

The accumulation of tubal secretions in the oestrous rabbit (Black & Asdell, 1959) sheep (Edgar & Asdell, 1960a) and cow (Black & Davis, 1962) following ligation at the ovarian end introduced further supporting evidence of an occlusive mechanism. While Black & Asdell (1959) failed to find histological evidence of their proposed oestrogen-induced oedema at the utero-tubal junction, limited evidence was later obtained with the ewe (Edgar & Asdell, 1960a). In contrast, removal of the utero-tubal junction and part of the isthmus, or the application of nicotine to these regions did not reduce the fluid distension in the cow (Black & Davis, 1962). Whether the fluid is eventually released into the uterus by a diminution of the oedematous condition or a relaxation of the isthmic musculature was not resolved.

In support of these findings, Stavorski & Hartman (1958) while carrying out an extensive investigation using utero-tubal insufflation, found the isthmus to be more important than the utero-tubal, or isthmic-ampulla junction, in resisting the passage of gas.

It is thus possible that the constriction of the isthmus is only a function of its small diameter and convoluted nature although a full explanation is still required for the cyclic nature of the phenomenon.

E. PURPOSE OF THE INVESTIGATION

It is apparent that the Fallopian tubes are highly specialised organs with distinct anatomic regions intergrated in function so as to move ova and spermatozoa in contrasting directions during similar periods in time. The part played in these advents by the muscular and ciliary activity of the tube has received considerable attention in the past. In contrast, knowledge on the movement of fluid secreted into

the lumen of the female genital tract is extremely limited. Since Woskressenski in 1891 postulated the presence of an active secretion in the Fallopian tube of the rabbit emphasis has been on investigating the role of genital fluids as media through which early metabolic and developmental changes of ova and spermatozoa may be prompted (Mastroianni, 1962). Thus the quantity of fluid secreted and changes in some of its components during the oestrous cycle have been investigated only in a limited number of species.

The direction of flow of tubal secretions, and their possible role as a medium in which ova and spermatozoa are conveyed, do not appear to have been measured quantitatively. This investigation was therefore primarily concerned with devising, and testing, a method which would allow continuous collection and quantitative assessment of the direction of fluid flow from the Fallopian tube in the ewe. Two parameters, flow through the ovarian end of the ampulla and through the utero-tubal junction into the uterus, were to be measured in the same animal on successive days of the normal oestrous cycle. Summation of these two parameters would provide information on the quantity of fluid secreted by the Fallopian tube.