Gamete Transport and Its Implication on the Proper Technique for Artificial Insemination*

Jose Arceo N. Bautista, DVM, Ph.D.**

Introduction

Artificial Insemination (AI) has been with us since the early 1900's. This technology, coupled with the advancement in semen cryopreservation in the late 1940's, has revolutionized the field of animal breeding. AI has been used in a variety of ways including the formation of synthetic breeds, the improvement of local breeds whether for beef or dairy, the establishment of exotic purebred lines in alien environments without live importation and many more. In 1964, the National Artificial Breeding Center (NABC) was established in the Alabang Stock Farm, Alabang, Rizal. A joint project between the Bureau of Animal Industry and the Dairy Training and Research Institute, the NABC endeavored to establish AI as a tool for the improvement of our indigenous breeds towards developing the local beef and dairy industry. A significant component of this strategy was the placement of AI centers in every province of the country under the supervision of the Bureau of Animal Industry, Department of Agriculture and Natural Resources. Likewise, this required the placement of able AI technicians in these centers, catering to the breeding needs of the livestock population in the province. To satisfy the demand for AI technicians, the NABC embarked on the training of personnel from the various stock farms under BAI and some academic institutions, in the then relatively new field of Artificial Insemination.

In the field of Reproductive Physiology, much has changed particularly in the understanding of the cellular and molecular mechanisms involving reproduction in the last 10-15 years. In this period, we have known that follicular dynamics in cattle occur in waves. A pattern which would lead us to understand the mechanism of why we are able to induce folliculogenesis in the luteal phase with the use of exogenous hormones. The clearer mechanism of gamete transport has also been understood, changing the techniques in AI that are employed in the field, particularly the proper placement of semen, proper dosing of semen, timing of AI and many more. It is in this context therefore, that these developments are brought to fore to educate you, the cattle raisers, on the updates in gamete transport, with the hope that these new knowledge can lead to better efficiency with the use of AI, and hopefully, for the AI practitioners among you, to discard the bad habits that you have so wantonly believed to be effective.

Gamete Transport Mechanism

A. Sperm Transport in the Male Reproductive Tract

As sperm cells are produced in the seminiferous tubules of the testes, these immature cells move from the lumen of the tubules to the head (caput) epididymides of both testicles via the respective rete testes and vasa efferentia. The transport of sperm is largely characterized by fluid flow and aided by ciliary movement in the vasa efferentia (see Fig.1). As the spermatozoa flow to the epididymides, they begin to acquire the ability to fertilize and become motile. However, from the vasa efferentia to the epididymis, fluid is also absorbed, thus, increasing the density of the sperm cells that are finally stored in the tail of the epididymis until ejaculation (see Fig.2). While the seminiferous tubules produce about 1-25 billion sperm cells a day, the concentration of sperm stored in the tail of the epididymis increases to 10-50

*Paper presented at the 2014 FCRAP Convention/General Assembly, Tuguegarao City, January 31, 2014.
**Associate Professor, Animal and Dairy Sciences Cluster, College of Agriculture, University of the Philippines at Los Banos, College, Laguna

billion, which is good for 5-10 ejaculates. In the length of the epididymis, no protein synthetic activity occurs (meaning no DNA synthesis), there is addition or loss of plasma membrane proteins and lipids, there is redistribution of proteins and lipids within the sperm cell, and changes in lipid diffusion coefficients.

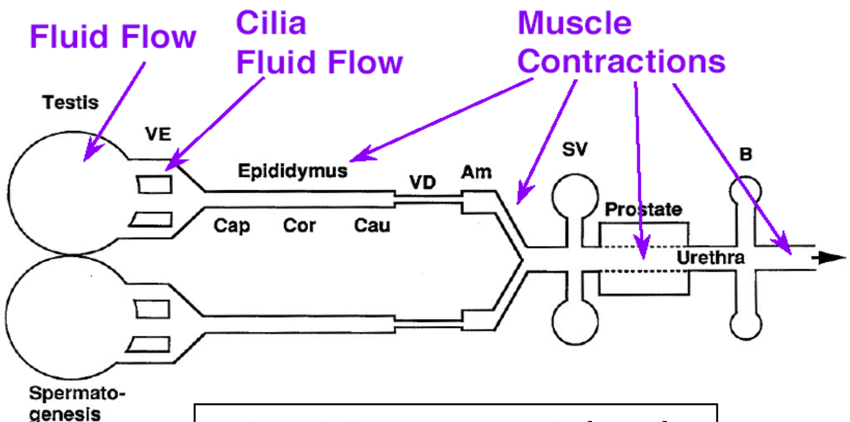
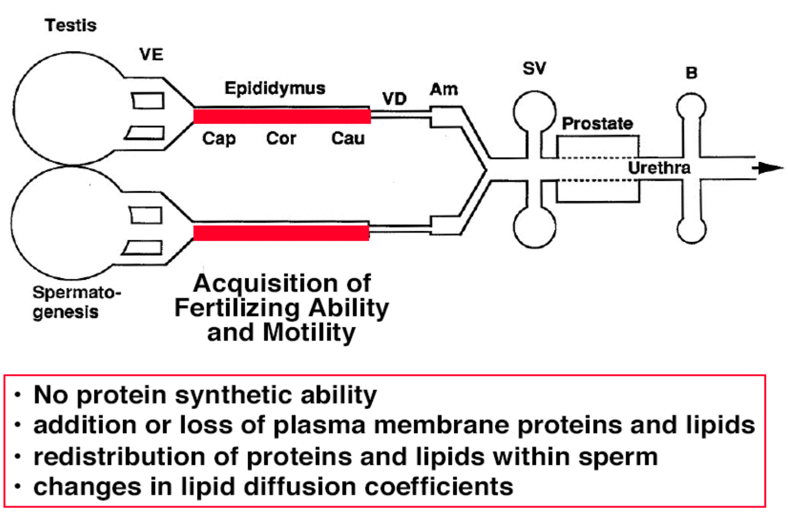


Figure 1. Sperm Transport in the Male Reproductive Tract

Figure 2. Changes in the Spermatozoa in the Epididymis

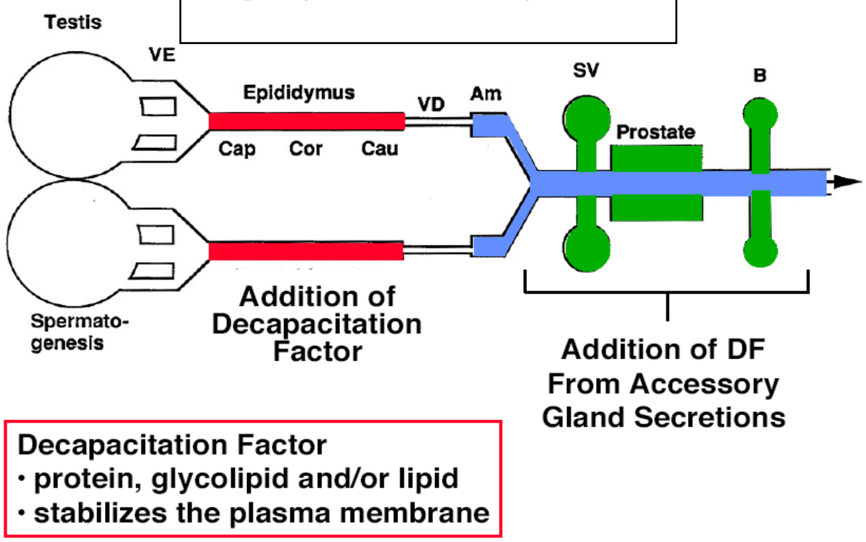


While the ability to fertilize and become motile is achieved in the epididymis, the sperm cells continue with these processes to the point that these lead to sperm capacitation, thus, exhausting all energy that is stored in the sperm cell even before these are ejaculated. In order to prevent this from

*Paper presented at the 2014 FCRAP Convention/General Assembly, Tuguegarao City, January 31, 2014.
**Associate Professor, Animal and Dairy Sciences Cluster, College of Agriculture, University of the Philippines at Los Banos, College, Laguna

happening, a Decapacitation Factor (DF) is added to the volume of the sperm cells in the length of the epididymis in most animals (see Fig. 3). Also, some DF is added to the sperm cells from the secretions of the accessory sex glands.

Figure 3. Addition of Decapacitation Factor (DF) to the sperm in the Epididymis and Accessory Glands



So what is the DF made of and what does it do to the sperm cell? The DF is either a protein, a glycolipid (carbohydrate-containing lipid) and/or a lipid. It prevents the processes in the sperm cell from progressing to capacitation while still inside the male reproductive tract, thus ensuring that sperm cells that are ejaculated have plenty of energy reserves, have not deteriorated and are ready to face the harsh environment and perils of transport in the female reproductive tract.

B. Emission, Ejaculation and the Site of Semen Deposition

Table 1 summarizes the different placements of the ejaculated sperm (this time properly termed semen) in different animal species.

Site of ejaculation	Semen Characteristics	Species
Vagina	slight coagulation of ejaculate	human, rabbit
vagina	semen with high sperm concentration	cattle, sheep
uterus	voluminous, distention of cervix	horse
cervix, uterus	voluminous, retention of penis during copulation	dog, pig
uterus	vagina plug	rodents

*Paper presented at the 2014 FCRAP Convention/General Assembly, Tuguegarao City, January 31, 2014.
**Associate Professor, Animal and Dairy Sciences Cluster, College of Agriculture, University of the Philippines at Los Banos, College, Laguna

Emission is the movement of sperm cells from the tail of the epididymis (where it is stored), up the vas deferens, to the ampulla and finally to the common ejaculatory duct (colliculus seminalis). This movement is aided by muscular contractions from the tail of the epididymis to the ampulla by the contraction of smooth muscles along the length of these parts of the male reproductive tract (see Fig. 1).

Ejaculation is the movement of sperm cells from the colliculus seminalis (common ejaculatory duct) to the urethra and out the male reproductive tract. This process also results into the increase in the volume of the ejaculate by the addition of fluids secreted by the accessory sex organs. The moment that secretions from these glands are added to the sperm cells, the mixture will now be called semen. The ejaculatory process happens upon sufficient stimulation at the time of coitus or mating. The process can be explosive at times due to the fact that strong muscles surround the urethra at the base of the penis, particularly the bulbospongiosus muscle which squeezes part of the bulbo-urethral gland at the time of ejaculation.

Generally, the semen is deposited in the vagina of the female in most animal species. However, species differences exist as to the placement of the ejaculated semen (see Table 1).

C. Sperm Loss in the Female Reproductive Tract

The moment that semen is deposited in the female reproductive tract at the time of coitus, the sperm cells are subjected to a coterie of harsh, fatal and inhospitable environments and factors. It is expected therefore, that a large part of the population of sperm cells will be lost, destroyed or phagocytosed in the female reproductive tract. Up to what extent does the loss of sperm cells occur?

Generally, there are 2 processes that occur which end up in significantly diminishing the population of sperm cells in the female reproductive tract. These are: retrograde flow and phagocytosis by neutrophils.

Retrograde flow (backflow) is characterized by the presence of sperm cells in the vagina after significant time has elapsed post-coitus or semen deposition. In experiments done in the cow, three (3) sites of semen deposition were performed by a group of seasoned AI technicians (the normal target of semen deposition is the body of the uterus, but was revised only for this experiment) on animals in heat. These sites were: the cervix, the body of the uterus and the horn of the uterus (see Fig. 4). Retrograde flow was assessed by recovering sperm in the vagina 1-8 hours after insemination. The results are shown in Fig. 5 and Fig. 6.

The recovery of sperm from the vagina of cows inseminated in the horn and body of the uterus showed that around 18% of the inseminated sperm underwent retrograde backflow. No significant statistical difference was observed between the results of inseminations in the body of the uterus with those in the uterine horn (Fig. 5). However, comparison of the results of inseminations done in the cervix with those in the uterine horn has shown significant differences (Fig. 6). Almost 60% retrograde flow in the inseminations done in the cervix was recorded while those in the uterine horns had the highest at only 28% after 8 hours. The results confirm that as far as retrograde flow is concerned, the body of the uterus is the correct site for the deposition of semen in artificial insemination.

So how do animals compensate for this loss of sperm cells by retrograde flow?

*Paper presented at the 2014 FCRAP Convention/General Assembly, Tuguegarao City, January 31, 2014.
**Associate Professor, Animal and Dairy Sciences Cluster, College of Agriculture, University of the Philippines at Los Banos, College, Laguna

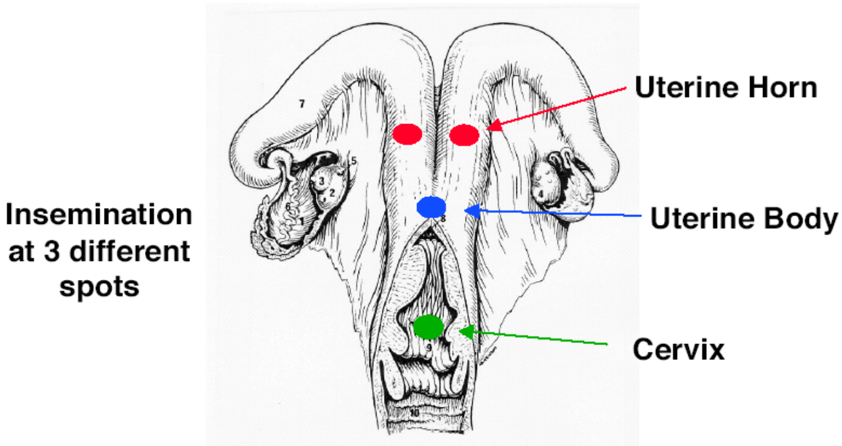


Figure 4. Insemination Sites Used in the Experiment

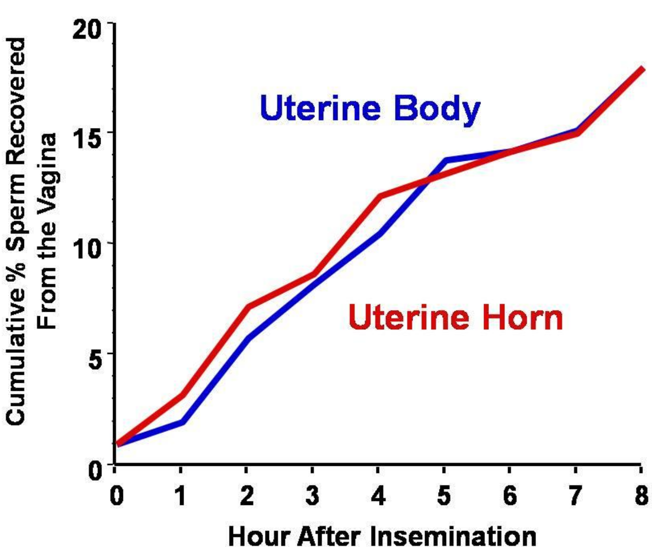


Figure 5. Recovery of Sperm from the Vagina

*Paper presented at the 2014 FCRAP Convention/General Assembly, Tuguegarao City, January 31, 2014.
**Associate Professor, Animal and Dairy Sciences Cluster, College of Agriculture, University of the Philippines at Los Banos, College, Laguna

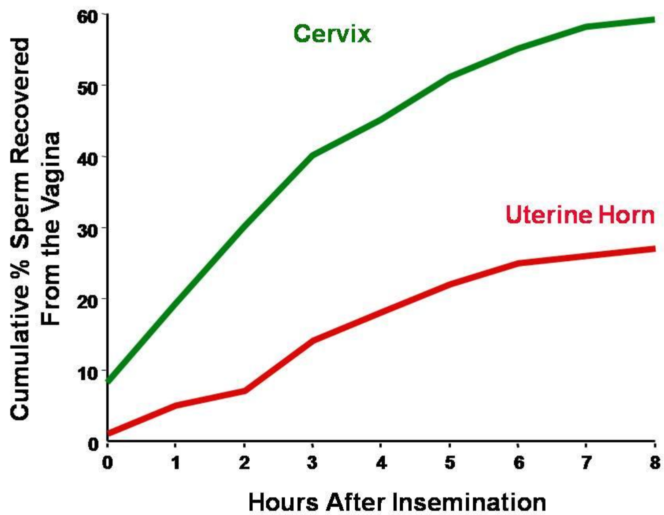


Figure 6. Comparison of Recovery Rates Between Uterine Horn and Cervical Depositions

Table 1 shows the characteristics of semen as deposited in the female reproductive tract in different animal species. Compensatory mechanisms exist with regard to sperm loss due to retrograde flow. These are summarized as follows: coagulation of semen or the formation of a plug as in humans and rodents; concentrated semen as in some small ruminants (sheep and goats), and the deposition of semen in the uterus as in horses and pigs. All of these compensatory mechanisms exist as evolutionary strategies of the different species for the perpetuation of their kind.

At the time of heat or estrus, the effects of the increased levels of estrogen are still felt by the system. Estrogen effects include reddening of the vulva, behavioral changes, swelling of the vulva, increased blood flow to the reproductive tract and increased (upward) contractions of the uterine lining. The latter (uterine contractions) is interpreted as uterine tone by most AI technicians inseminating truly in-heat cows. Increased flow of blood to the reproductive tract means there is enough population of blood components flowing to the uterus. At the time of coitus and deposition of semen in the vagina, the increased blood flow predisposes the sperm cells to the increasing number of neutrophils already present in the uterine tract. Contrary to what some believe that the cow's neutrophils are selective in performing their phagocytic function as a first line of defense to microbial infection, sperm cells are considered to be alien invaders to the female reproductive tract, hence, are also subjects for phagocytic action by these neutrophils. Phagocytic action of sperm cells by neutrophils in the uterine lining effectively diminishes the population of sperm cells that eventually reach the oviduct, particularly the isthmus, where they lodge in wait for the coming of the ovulated egg. The timing of neutrophil invasion after heat and insemination is shown in Fig. 7.

*Paper presented at the 2014 FCRAP Convention/General Assembly, Tuguegarao City, January 31, 2014.
**Associate Professor, Animal and Dairy Sciences Cluster, College of Agriculture, University of the Philippines at Los Banos, College, Laguna

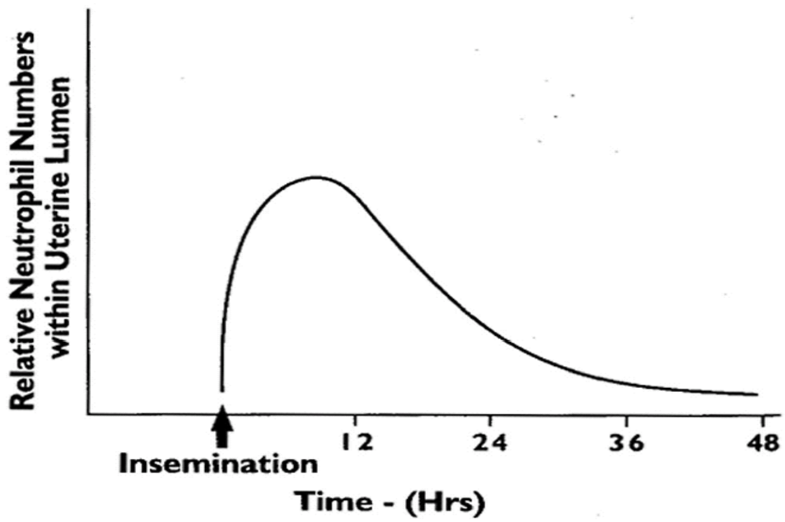


Figure 7. Infiltration of the Uterus with Neutrophils

As shown above, infiltration of the uterine lumen by neutrophils occurs right after semen is deposited into the vagina (as in coitus) or after artificial insemination in the uterine body. There is a tremendous increase in neutrophil infiltration within 12 hours (peaks from 10-11 hours), thus within this period, sperm cells that venture close to the uterine lining are at the mercy of the neutrophils and are likely to be phagocytosed. So what is the implication of this knowledge to AI?

Some bad habits practiced by AI technicians, such as the improper placement or deposition of semen in the cervix, double dosing of semen and follow up insemination 12-24 hours later, are taking out the science in the technology of AI in the bovine. In the case of neutrophil infiltration after insemination, it is implied that because of increased phagocytic activity, follow up inseminations are useless, unnecessary and wasteful.

D. Phases of Sperm Transport

Contrary to popular belief that the moment semen is deposited in the vagina, there is a mad rush to the oviduct by the sperm cells in search of the egg, and whoever gets to the egg first is the lucky one that fertilizes it. In the first place, the deposition of semen happens only at the time of estrus or standing heat (lordosis) as in coitus, or even as late as the end of heat (as in AI). At this time, there is still no ovulation, therefore, no egg to be found. Ovulation in the cow occurs around 11 hours or half a day after the end of heat. So what happens to the sperm cells that were deposited either in the vagina (coitus) or in the body of the uterus (AI)?

The fate of the sperm cells are shown in Fig. 8. There are three (3) transport mechanisms that have been identified. These are: rapid transport mechanism, sustained transport mechanism and oviductal transport.

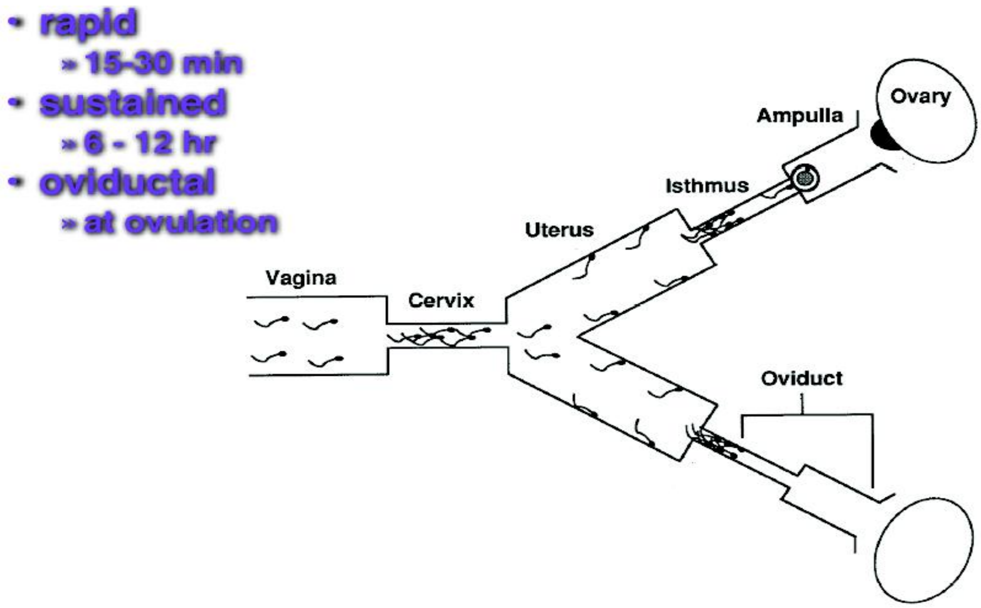


Figure 8. Phases of Sperm Transport

In the rapid transport mechanism, sperm cells are found in the oviducts as early as 15-30 minutes after insemination. From the vagina, these sperm cells are able to move up, cross the cervix, traverse the length of the uterus and enter the oviducts. For its size, the sperm cell must be pretty fast to be able to reach the oviduct in such a short time. So how is this possible?

At the time of standing heat (the only time when the cow accepts the bull to mount her), upward contractions of the uterine muscles occur. These contractions propel the sperm cells towards the oviducts, as in a surfer riding the wave. Studies have shown that because of this mechanism aided by upward uterine contractions, sperm cells reach the oviduct in such a short time. The same studies however, point out that the sperm cells do not only reach the oviducts but also cross it, and some even spill into the peritoneal cavity. Further studies have also pointed out that most of the sperm cells that reach the oviducts at this stage, are the kinds that have no ability to fertilize an egg: dead, non-motile and morphologically abnormal sperm cells. The rapid transport mechanism must therefore, serve a purpose for eliminating the unwanted portion of the sperm population, but never for producing the lucky sperm that will eventually fertilize the egg. So where does the lucky fertilizing sperm cell come from?

Morphologically normal sperm cells deposited in the vagina that move up on their own strength, must be able to survive the inhospitable environment from the vagina to the oviducts. This also means that the sperm cells that eventually reach the oviducts are the ones that have survived being lost in the crypts of the cervix and to the wrong uterine horn, were not washed out and neutralized by mucus secretions specially in the cervix, evaded the phagocytic neutrophils in the uterus, and settled in the lining of the isthmus of the oviduct in 6-12 hours. This is the sustained transport mechanism (see Fig. 8). Experiments were done to prove this mechanism and its relation to fertilization of the ovulated egg (see Fig. 9 and Table 2). Sperm cells that survive this journey, settle in the isthmus portion of the oviduct where they attach to the supporting cells that line this part of the reproductive tract, and lie in wait for the coming of the ovulated egg. These support cells maintain the integrity and viability of the sperm cells up to 3 days.

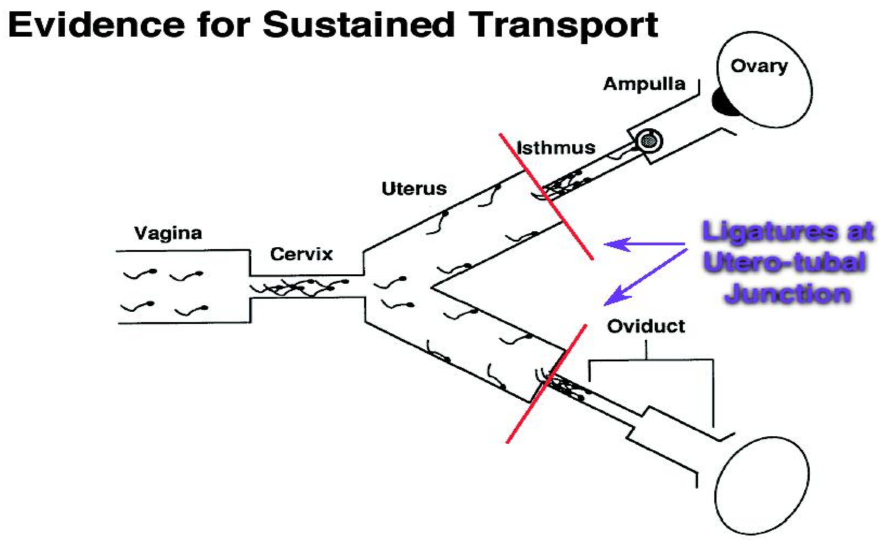


Figure 9. Experiments Done to Prove the Sustained Transport Mechanism

In the experiments conducted to prove that sustained transport occurs, transection (ligature) of the utero-tubal junction was performed on experimental in-heat heifers at several hour-intervals (6, 8 and 12 hours) after insemination. The experiments also showed how many of the ovulated eggs were fertilized after the ligature (see Table 2).

Effect of Oviduct Transection on Fertilization in Heifers

Interval from mating* to transection of UTJ	Oocytes Fertilized
6	0%
8	50%
12	83%

*** Mating was done at the start of estrus.**

Table 2. Results of the Experiment on Sustained Transport Mechanism

The results shown in Table 2 prove that up to 6 hours post-mating, when transection was performed in mated heifers, no ovulated eggs were fertilized. This only means that at the time of ligature,

*Paper presented at the 2014 FCRAP Convention/General Assembly, Tuguegarao City, January 31, 2014.
**Associate Professor, Animal and Dairy Sciences Cluster, College of Agriculture, University of the Philippines at Los Banos, College, Laguna

the fertilizing sperm cell has not effectively travelled beyond the utero-tubal junction to meet the egg. When the ligature was performed at 8 hours, some 50% of the ovulated eggs were fertilized, and at 12 hours, 83% of ovulated eggs were fertilized. This experiment proved that the fertilizing sperm cell needed at least 6-12 hours to swim up to the oviduct in order to successfully fertilize the ovulated egg, hence, the importance of the sustained transport mechanism in the fertilization of the (ovulated) egg. Similar experiments were also conducted in pigs (see Fig. 10).

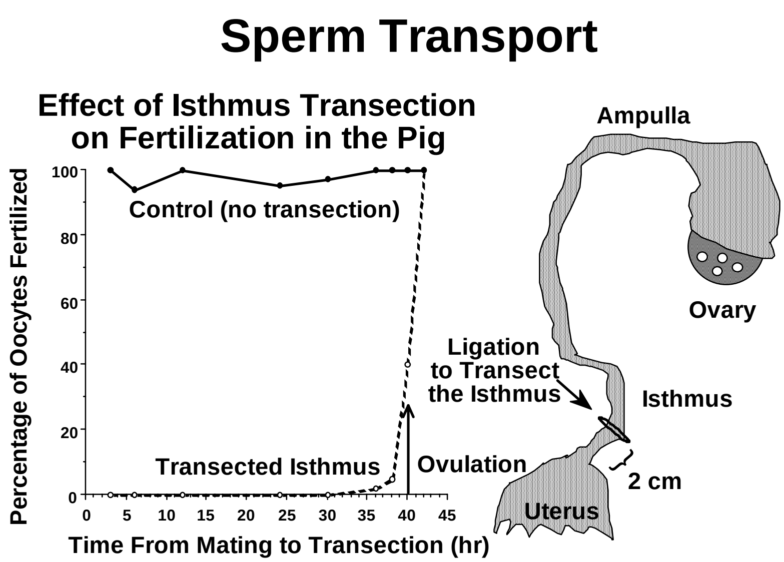


Figure 10. Sperm Transport in the Pig

In the pig (a multiple ovulator), transection of the isthmus was performed 2 cm above the utero-tubal junction at 3, 6, 12, 24, 30, 40 hours after mating. Up to 30 hours, no fertilized eggs were seen in the oviducts, while in the non-ligated control, almost all of the eggs were fertilized. After 30 hours, some eggs were fertilized but at 40 hours, almost all of the ovulated eggs were fertilized similar to the non-ligated control, again proving that in other species, the sustained transport mechanism works in a similar fashion as in cattle.

So what happens to the sperm cells that reach the oviduct through the sustained transport mechanism? The sperm cells that traverse the uterus into the isthmus attach to the lining of the oviduct and lie in wait for ovulation to occur. Apparently, the attached sperm cells are nourished by the support cells found in the lining. When ovulation of the egg begins, a factor present in the blood triggers the processes leading to ovulation. This same factor is the very factor that triggers some (very few) sperm cells to detach from the isthmic lining and start to swim up the oviduct to meet the egg. This is oviductal transport . The intricacy of the sharing of the same factor that triggers ovulation and the signal for the sperm cell in the isthmus to swim up in oviductal transport is explained by the unique blood supply to the ovary and the oviduct (see Fig. 12). Oviductal transport occurs as early as 1-2 hours before the egg is finally ovulated (see Fig. 13), and caught by the fimbria. When the egg is ovulated, it enters the oviduct

*Paper presented at the 2014 FCRAP Convention/General Assembly, Tuguegarao City, January 31, 2014.
**Associate Professor, Animal and Dairy Sciences Cluster, College of Agriculture, University of the Philippines at Los Banos, College, Laguna

by way of the ostium and infundibulum, finding its way to the ampulla where it finally lodges at the ampullary-isthmic junction (the egg cannot pass through this junction at this time, because of the smaller diameter of the isthmus). At this ampullary-isthmic junction where the egg is lodged, sperm cells from the oviductal transport mechanism meet the egg to fertilize it.

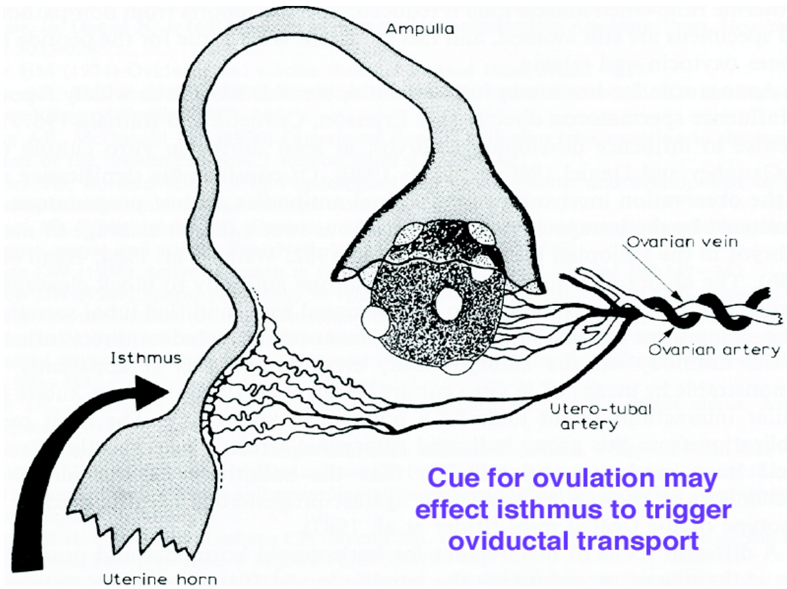


Figure 11. Unique Blood Supply of both Ovary and Oviduct

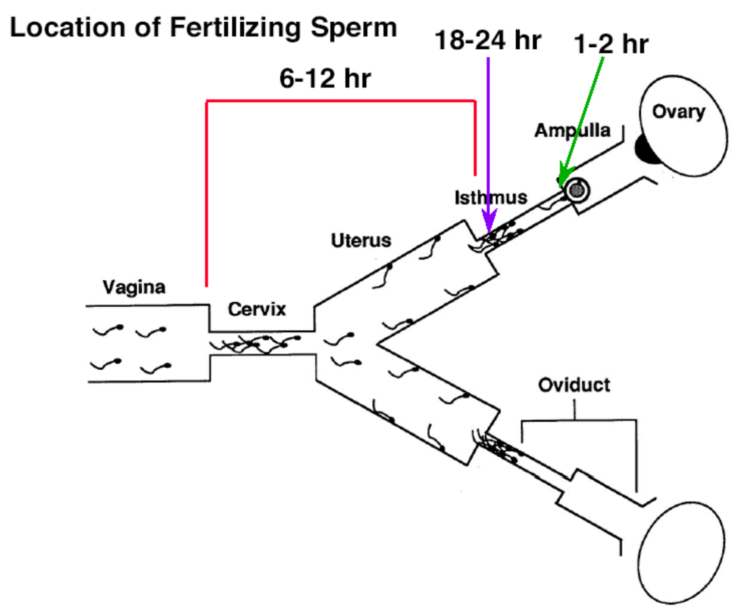


Figure 12. Location of the Fertilizing Sperm in Oviductal Transport

*Paper presented at the 2014 FCRAP Convention/General Assembly, Tuguegarao City, January 31, 2014.
**Associate Professor, Animal and Dairy Sciences Cluster, College of Agriculture, University of the Philippines at Los Banos, College, Laguna

Ovulation and Transport of the Egg

Ovulation in the cow is not an explosive event as some movies would depict. A picture of an ovulated egg together with the cells surrounding it and some fluids is shown in Fig. 13.

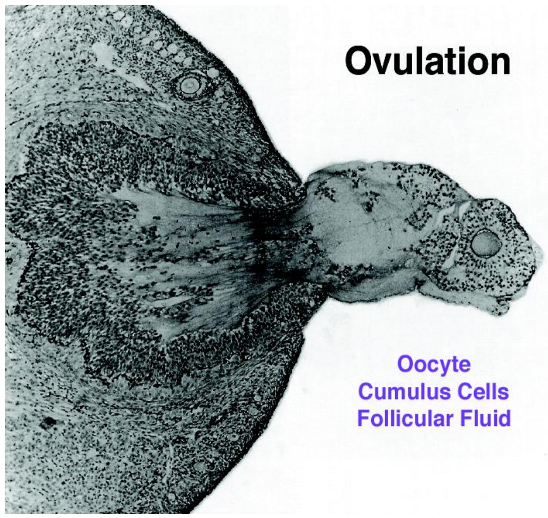


Figure 13. Ovulation in the cow

When the egg is ovulated, it is still surrounded by layers of cumulus cells that somehow protect it, together with follicular fluid that may be rich in estrogen. In the oviduct, the ampulla hosts the ovulated egg; its diameter as large as the egg itself. However, the isthmus of the oviduct is smaller in diameter than the ampulla so that at the junction of the ampulla and the isthmus (aptly called the ampullary-isthmic junction), the egg lodges and becomes immobilized. This is the chance for the sperm cells to meet the egg and fertilize it, as it would be easier to penetrate the egg when it is immobile, thus, the ampullary-isthmic junction plays a big role in the successful fertilization of the egg. In other words, the site of fertilization of the egg is the ampullary-isthmic junction.

Getting to the ampullary-isthmic junction however, is no easy task for the ovulated egg. It has to be caught by the fimbria, enter the ostium (opening of the oviduct) and traverse the infundibulum to the ampulla while going against the flow of fluids from the oviduct (see Fig. 14). Just how much fluid flows out of the oviduct? Experiments have shown that significant quantities of fluids are secreted by the oviduct (see Fig. 15). About 2.0 ml of fluid in a 24-hr period has been measured in the oviduct around the time of heat and ovulation. This volume of fluid flow wanes at around the time that a functional corpus luteum is formed (around 5-7 days) and increases again around the time of the next heat. When further experiments were conducted to measure the directional flow of fluids in the oviduct, researchers have found that a total of 1.25 ml of fluids flowed from the oviduct around 2 days after heat. Of this, only 0.25 ml flowed to the uterus, while around 1.0 ml flowed outwards to the ampulla (see Fig. 16).

*Paper presented at the 2014 FCRAP Convention/General Assembly, Tuguegarao City, January 31, 2014.
**Associate Professor, Animal and Dairy Sciences Cluster, College of Agriculture, University of the Philippines at Los Banos, College, Laguna

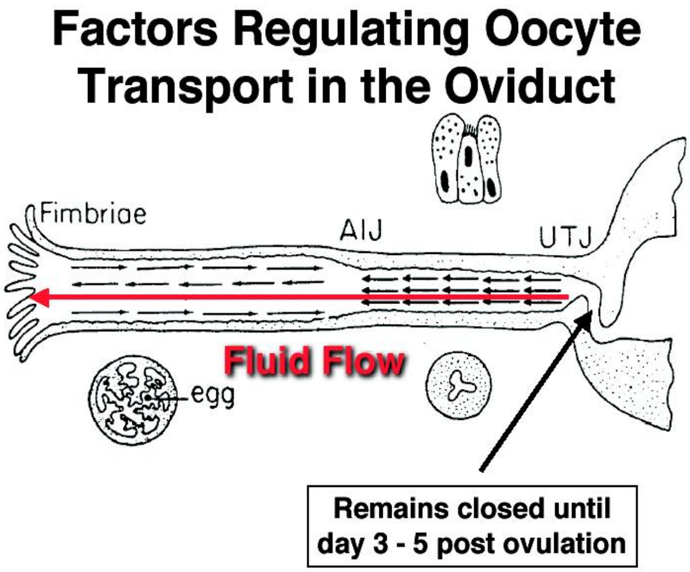


Figure 14. Fluid flow in the Oviduct at the Time of Ovulation

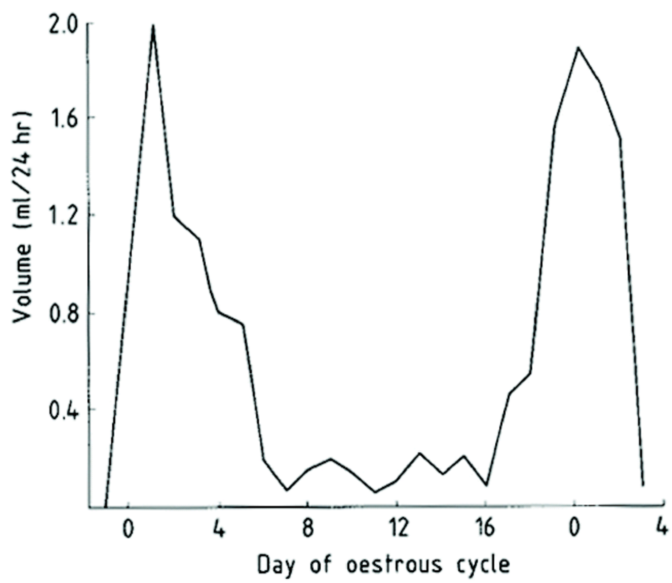


Figure 15. Volume of fluid in the Oviduct at different stages of the Estrous Cycle

*Paper presented at the 2014 FCRAP Convention/General Assembly, Tuguegarao City, January 31, 2014.
**Associate Professor, Animal and Dairy Sciences Cluster, College of Agriculture, University of the Philippines at Los Banos, College, Laguna

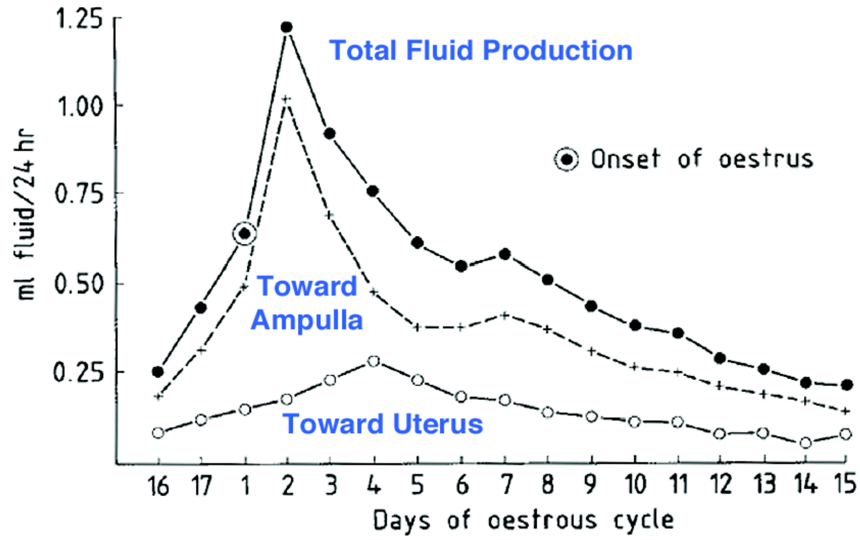


Figure 16. Determination of Directional Fluid Flow in the Oviduct

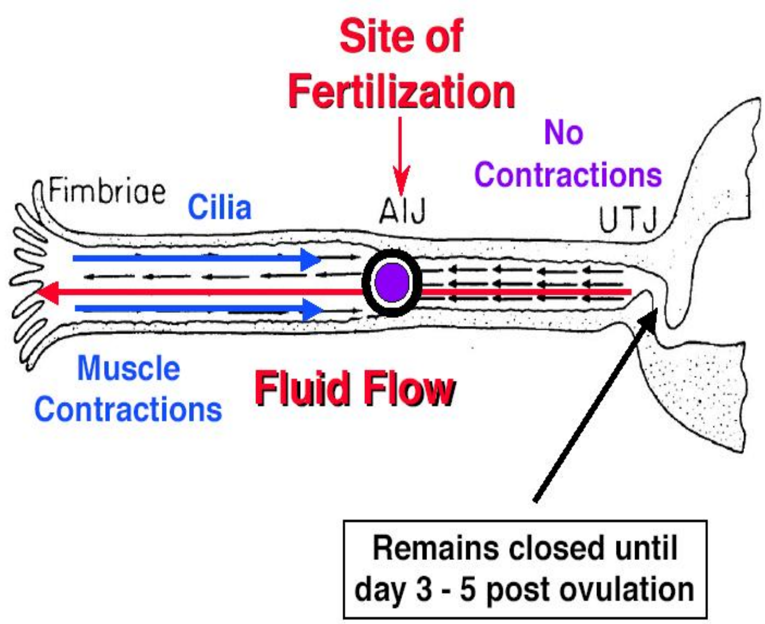


Figure 17. The Oviduct at the Time of Fertilization

*Paper presented at the 2014 FCRAP Convention/General Assembly, Tuguegarao City, January 31, 2014.
**Associate Professor, Animal and Dairy Sciences Cluster, College of Agriculture, University of the Philippines at Los Banos, College, Laguna

When the egg finally traverses the ampullary region of the oviduct and reaches the ampullary-isthmic junction, it is prevented from moving further by the smaller diameter of the isthmus. This is also about the time that the sperm cells that have moved up to meet the egg by oviductal transport finally meet the egg (see Fig. 17). Sperm cells have to penetrate the layer of cumulus cells that surround the egg to be able to attach to the zona pellucida. This is possible only if the sperm cells have undergone capacitation, when, through the acrosome reaction, the enzymes acrosin and hyaluronidase are released. Hyaluronidase is an enzyme that breaks the bonds of the cumulus cells, thus, parting a path for the sperm cell to move closer to the egg. When the sperm cells attach to the zona pellucida, the process of fertilization begins.

After fertilization, the zygote (fertilized egg) undergoes pre-implantation development still inside the oviduct. The first cleavage begins around Day 2 (62 hours) when the zygote divides to form the 2-cell stage. This will be followed by more cleavage divisions.

While pre-implantation development of the early embryo occurs in the oviduct, changes are also happening at the ovary where the egg came from. In the cavity formed by ovulation, blood invades and clots, turning the structure into a corpus hemorrhagicum. As blood brings more nutrients to the structure, the left-over cells in the former follicle begin to grow into the cells that eventually become the progesterone-secreting cells of the corpus luteum. Progesterone production will be an important factor in changing the milleu in the oviduct. Because of the calming effect of progesterone on the reproductive tract, the muscles lining the isthmus relaxes in 3-5 days, increasing the diameter of the lower portion of the oviduct and allowing the early embryo to move downwards to the uterus. The final obstacle, the utero-tubal junction, similarly relaxes, allowing the early embryo to move to the uterus where it implants itself in around 17 days, establishing a pregnancy.

Summary

New developments in the field of reproduction have brought us a better understanding of the mechanisms that govern success and failures in the use of reproductive technologies. The information presented here, hopefully, will improve the knowledge of those engaged in animal production, particularly the very people who carry on the passion for cattle raising. Artificial Insemination may have been our best tool for genetic improvement, but we, the users of such technology must be ready to embrace new developments, new knowledge and skills, to increase our efficiency in carrying out the passion for cattle production. Finally, as a tribute to the new year, let us kick out the bad habits that have kept us in the dark through all these years, and that includes the unshakable habits we do in AI.

References:

1. Animal Science 434 Lecture No. 15, John Parrish. University of Wisconsin Madison, Wisconsin. 2007. Used with Permission from the author.
2. Pathways to Pregnancy and Parturition. P.L. Senger, 2nd Revised Ed. 2005, Current Conceptions, Inc., Washington State University Research & Technology Park, Pullman, Washington, USA.

*Paper presented at the 2014 FCRAP Convention/General Assembly, Tuguegarao City, January 31, 2014.

**Associate Professor, Animal and Dairy Sciences Cluster, College of Agriculture, University of the Philippines at Los Banos, College, Laguna

For Inquiries:

Send me an eMail- jobau57@yahoo.com

T H A N K Y O U !

*Paper presented at the 2014 FCRAP Convention/General Assembly, Tuguegarao City, January 31, 2014.
**Associate Professor, Animal and Dairy Sciences Cluster, College of Agriculture, University of the Philippines at Los Banos, College, Laguna