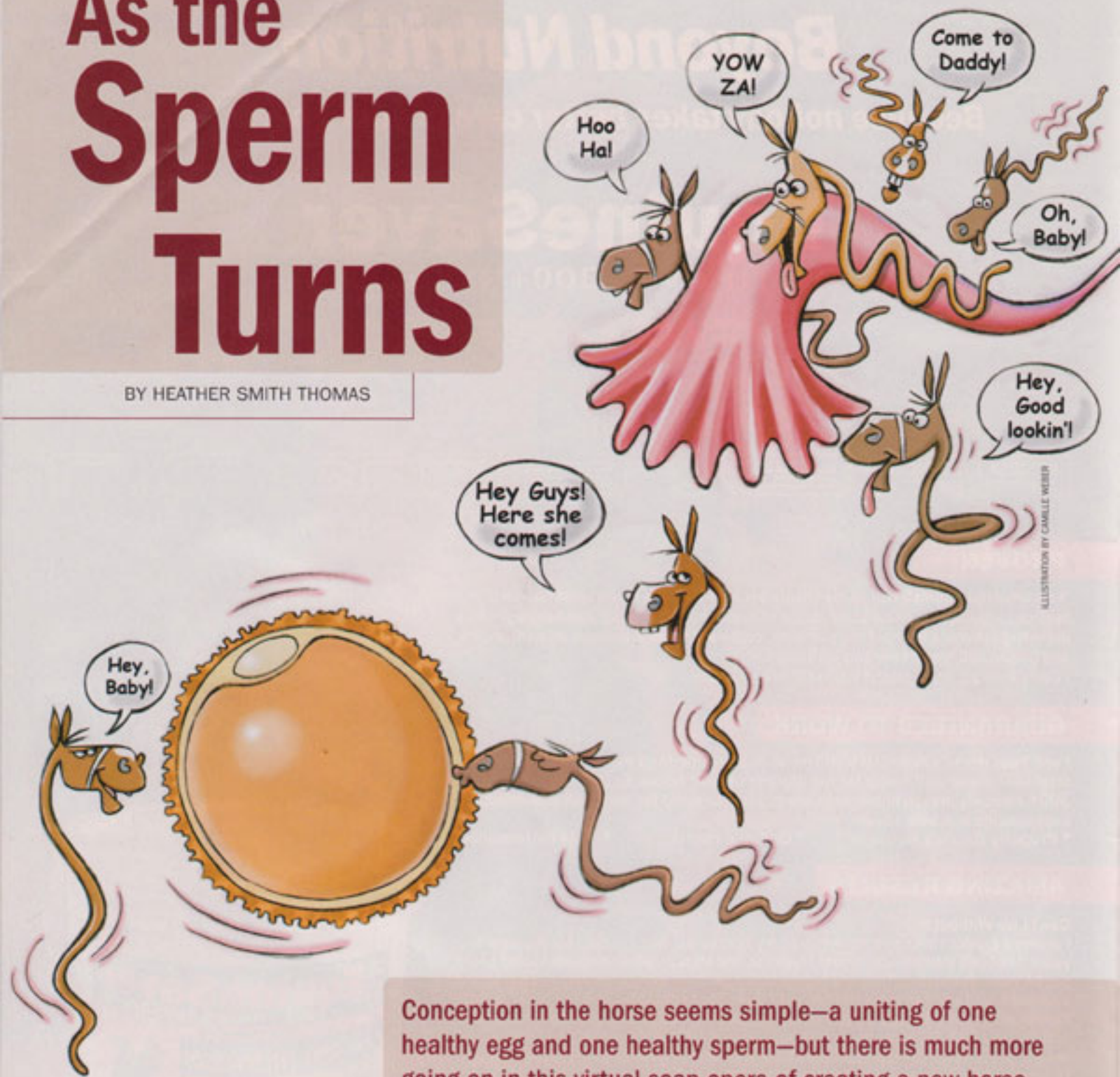


# As the Sperm Turns

BY HEATHER SMITH THOMAS



Conception in the horse seems simple—a uniting of one healthy egg and one healthy sperm—but there is much more going on in this virtual soap opera of creating a new horse

**F**ertilization is the beginning of a new individual: the culmination of many biological processes working in concert to form a new life. The timing and precision of it all is nothing short of a drama, playing out on a cellular level. In simple terms, a healthy mare produces an ovum (egg) in the core of her ovary. "The egg is delivered into the fallopian tube of the uterus, where it is

united with a sperm cell that has entered the mare's tract, or uterine tube, and found its way to the egg," says Woody Asbury, DVM, Dipl. ACT, retired dean of the College of Veterinary Medicine at the University of Florida. "The fertilized egg then travels to the uterus, where it will develop through the gestation period, nourished by fluids, then by vascular sources up until maturation and delivery."

Sperm cells are not as complex as their egg counterparts, but the method by which the two come together is a small miracle in itself. As explained by Ahmed Tibary, DVM, PhD, a professor of theriogenology in the Department of Veterinary Clinical Sciences at Washington State University, scientists are still learning about this very basic process, especially in terms of being able to help facilitate reproduction by manipulating semen. This includes using cool-shipped semen or frozen semen, *in vitro* fertilization, intracytoplasmic sperm injection, or other assisted reproductive techniques.

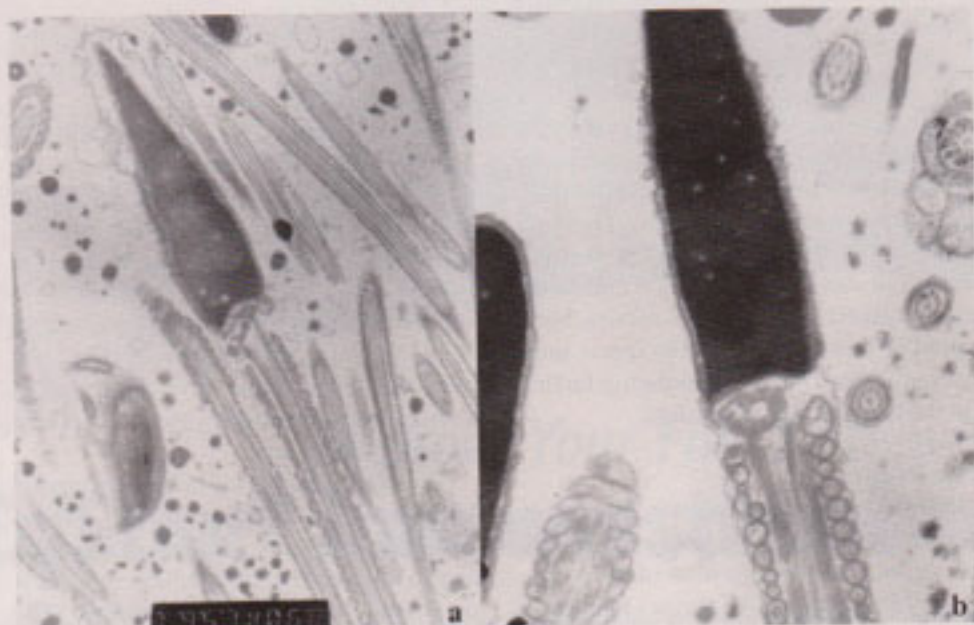
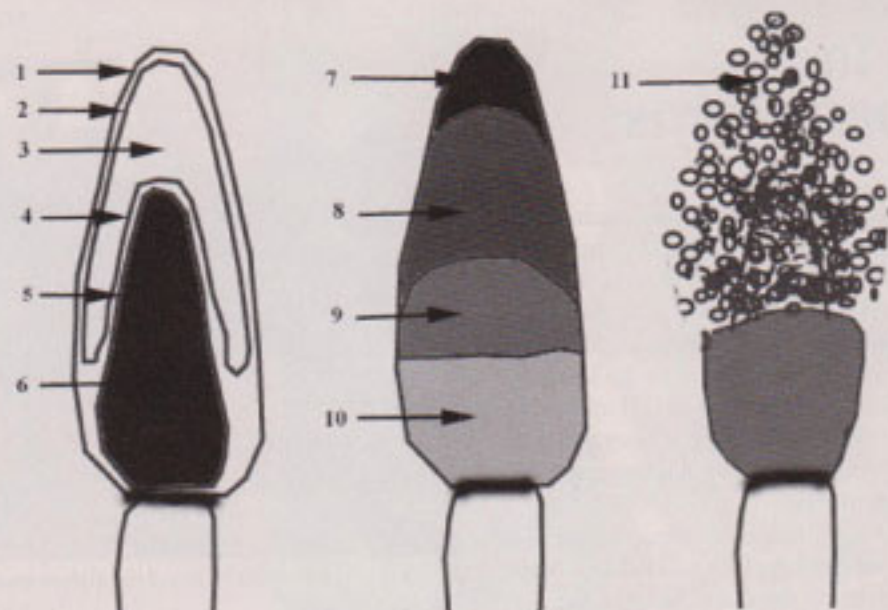
The process of fertilization and viability of spermatozoa with natural cover is one thing, but some of the changes that occur in the genital tract of the female to enable fertilization are hard to duplicate when we try to fertilize an egg outside the mare's body. Sometimes freezing and thawing—or even shipping—of semen can trigger changes, damaging sperm. This is why conception rate with these methods might not be as optimal for some stallions.

### A Look at Sperm

Ed Squires, PhD, formerly of Colorado State University and now director of advancement and industry relations and executive director of the Gluck Equine Research Foundation at the University of Kentucky, says semen must be deposited into the uterus to up the odds that it will encounter and unite with an egg. "In natural mating, the stallion's penis comes up against the mare's cervix and the end of the penis probably penetrates the cervix—particularly when the stallion is thrusting hard—to deliver most of the semen directly into the uterus," says Squires.

"With artificial insemination we try to deliver the semen into the uterus," he says. "Then by several different mechanisms the sperm make their way up into the opening of the oviduct (fallopian tube, alternately called the uterine tube). A lot of people think the sperm swim up there, which may be partially true, but they are aided by uterine contractions and fluid movement. This allows them to get to the tip of the (uterine) horn and the opening to the oviduct—the uterotubal junction."

Even though there might be more than 10 to 15 billion sperm deposited into the uterus in a stallion's ejaculate, only a few thousand of the little guys get into the oviduct. The egg needs a certain kind of sperm, though: "There is a selection process that occurs in the uterus so only the



The top illustration shows a schematic representation of acrosome reaction changes. Starting from left, a normal spermatozoon with anatomical regions: 1) plasma membrane; 2) outer acrosomal membrane; 3) acrosome content; 4) inner acrosomal membrane; 5) nuclear membrane; 6) nucleus; 7) apical region; 8) pre-equatorial region; 9) equatorial region; 10) post-equatorial region; and 11) fusion between plasma membrane and outer acrosomal membrane, resulting in vesiculation and release of enzymes (acrosome reaction). The bottom electron microscopy images show A) a sperm with intact acrosome; and B) a capacitated sperm.

most motile, morphologically normal sperm get there," Squires explains. "This sorting process is partly due to white blood cells (specifically, phagocytes) that clean up dead and dying sperm. Some of the sperm are expelled out through the cervix. A study at University of Florida showed that seminal plasma in some ways protects the good sperm and allows inferior ones to be more attractive to the white blood cells. Thus there is a selection/filtering process that occurs as the sperm are traveling through the body of the uterus." (This is why uterine inflammation occurs more often with insemination using semen

that has been frozen—the seminal plasma is spun down in a centrifuge and removed from the semen, thus, removing its protective effects.)

And so the proverbial pool of eligible sperm gets smaller. The most highly qualified spermatozoa enter the bottom part of the fallopian tube and colonize there in a sperm reservoir around the opening. They attach to the lining and rest, awaiting the egg's arrival. "This reservoir seems to be a preferred site where they can be nourished. As long as they are attached to the lining they seem to stay healthy," says Squires.

"In normal fertilization, there is a phase

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in which the spermatozoon needs to undergo some biochemical and morphological changes in order to be able to fertilize the egg," notes Tibary. The sperm are changing through this process, called capacitation, while attached to the lining.

It takes only one sperm cell to fertilize the egg. When you look at it from a biological standpoint, however, it takes more than one sperm to guarantee fertilization. To optimize the process, there must a population of available sperm candidates, if you will, present at the site of fertilization.

"When we inseminate a mare, we need a population of extremely good, viable, motile sperm cells," Tibary says. That small population is selected by process of elimination; the remaining sperm gather at the uterotubal junction. Those sperm cells are then released from the lining as the mare progresses through ovulation. Once released, they are programmed to fertilize the egg or die. Researchers don't know the mechanism that triggers this release, but it might be hormonal. Then the sperm must make the next move, progressing farther up the oviduct to meet the egg.

## The Ovum

The egg must be mature before it can be fertilized; therefore, timing of insemination in relationship to ovulation time is very important. "All those changes in the egg (that bring it to maturity) take place in the last few hours before ovulation," says Tibary. "After the egg has ovulated and is shed into the fallopian tube and carried along it, the egg continues to undergo changes that are critical to



With live cover, it's best to breed just before ovulation, so the sperm are ready and waiting for the egg to come along.

fertilization. When it gets to the fertilization site we hope there is already a sperm reservoir built up there, waiting."

In the ovary, the egg was in a fluid-filled follicle, which matures and ruptures explosively (Who says there isn't drama at the cellular level?). The egg is directed down into the fimbria, a fringe of tissue at the opening of the oviduct. "The fimbria is the outer portion, shaped like a catcher's mitt," explains Squires. It contains ciliated cells that create a wavelike motion to move the egg the proper direction, toward its conceptional fate.

The egg is small and sticky, surrounded by nurse cells called cumulus cells. "These make the total mass larger and allow the egg to stick to the cilia, which motion it down into the oviduct," says Squires. "The egg is viable for about six hours after ovulation, dying as it ages, so chances for fertilization are highest early on.

"If it does get fertilized late (an aged egg, fertilized after six hours or more), there's more risk for early embryonic loss;

the fertilized egg doesn't continue dividing," he explains.

## The Union

As mentioned previously, it's best to have sperm already in the tract at the time of ovulation, ready and waiting for the egg to come along," says Tibary.

"If the egg ovulates, then you try to breed the mare, conception rate drops," says Squires. Some people do this when using frozen semen, however; since they have a limited supply of semen. They wait until the mare ovulates, then put the semen into her within the first six hours. But with fresh or cooled semen, you want to have the sperm already there when the egg arrives.

"Capacitation defines a series of changes on the surface of the spermatozoa that allow the first step in the fertilization process," says Tibary. "If we have a mature egg, and enough of the capacitated spermatozoa, there is a good chance for fertilization." Following capacitation the spermatozoa in the running "acquire a hyperactive motility, allowing sperm-egg contact. They bind to the zona pellucida (a glycoprotein membrane surrounding the plasma membrane of the egg) and a second phenomenon—the acrosome reaction—allows the spermatozoa to provide the enzymes required to break through the outer envelope of the egg."

Even though there are several thousand sperm at the reservoir, by the time they get to the expectant egg there are only a few hundred left in line to fertilize. "When the ovum comes down to the site of fertilization, in the lower third of the tube, this is when all the chemical and physical interactions between the sperm and egg take

## VARIABLE VIABILITY OF SPERM

The amount of time that elapses between insemination, particularly if the mare is bred by natural cover, and the point at which there are no viable spermatozoa remaining in her tract to guarantee fertilization is quite variable. That time span depends on the individual stallion. "With natural cover, it's not unheard of for a mare that ovulates up to seven days after a single breeding to still conceive, if the stallion has long-lived sperm," says Ahmed Tibary, DVM, PhD, a professor of theriogenology in the Department of Veterinary Clinical Sciences at Washington State University. The difference in viability of sperm within the uterine tube—what makes some stallions more fertile than others—is something researchers are still trying to figure out.

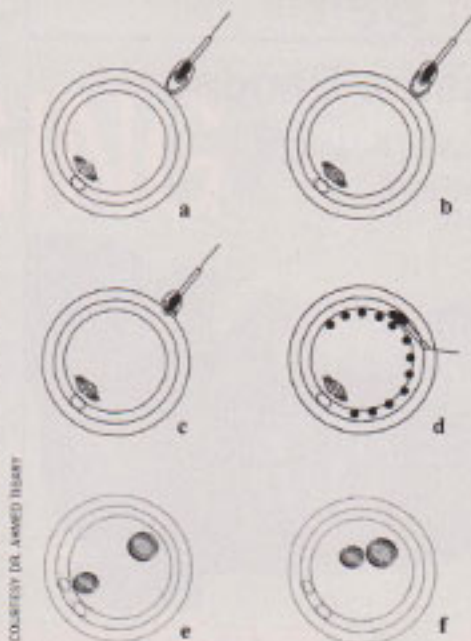
"In addition to individual variation, manipulation of semen (freezing and thawing, sex sorting, etc.) can induce capacitation-like changes which reduce viability of sperm and impose a very tight timing between ovulation and insemination," he explains. —Heather Smith Thomas

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place," says Tibary. "The first interaction is mostly chemical, and the second phase is physical, in which the sperm cells are trying to find their way through the envelopes of the egg. The reaction in the sperm is induced by contact with the zona pellucida at the outer surface of the egg, enabling it to penetrate. The zona pellucida processes create a specific binding site that can recognize the spermatozoon and bind to it. This is a highly specific mechanism; the equid egg can only recognize sperm from an equid (horse or donkey)."

After all of this tumult, one sperm emerges victorious. Once the spermatozoon has penetrated the zona pellucida, its nuclear membrane fuses with the egg's membrane. "That binding allows the sperm to be incorporated into the egg cytoplasm," says Tibary. "During this process of fusion and incorporation into the egg, another reaction occurs—the zona block (also called the zone reaction, the result of release of cortical granules, secretory vesicles residing below the egg plasma membrane)—so none of the other sperm can penetrate." This creates a barrier to keep the other sperm out, preventing polyspermy (more than one sperm penetrating the egg), which would kill the embryo.

"The first stage after one of them penetrates the egg involves chemical and biophysical changes of the sperm head,



**Sperm-egg interaction during fertilization.** a) binding to the zona pellucida (ZP); b) acrosome reaction; c) penetration of the ZP; d) binding to the vitellin membrane, fusion and cortical reaction; e) decondensation of the sperm nucleus formation of second polar body; and f) male and female pronuclei.

which cause the sperm cell to reorganize into a male pronucleus," he continues. "The fertilized egg becomes a zygote—when there are two pronuclei, one male and one female. They merge to form a diploid cell."

Chromosomes from the male and female join together into the zygote or single-cell embryo, and division of the cell begins. The first division occurs about 18 hours after fertilization. The cells then continue to divide within the uterine tube.

## ASSISTED REPRODUCTION

"There are mysteries we have not been able to figure out in horses," says Ahmed Tibary, DVM, PhD, a professor of theriogenology in the Department of Veterinary Clinical Sciences at Washington State University. "The equine is one species in which complete production of embryos by *in vitro* (in the lab) fertilization and *in vitro* culture has not been very successful. We have not been able to duplicate the reproductive tract environment. There have only been two foals produced by completely *in vitro* production of blastocysts. You can fertilize the egg *in vitro*, but in order for it to develop you must put it into the uterine tube immediately. Otherwise it stops developing."

In contrast, with other species, *in vitro* fertilization can be accomplished and the embryo grown for about six days *in vitro*, at which time you can put it directly into the uterus.

Knowing how conception takes place in the horse helps us understand importance of timing when breeding mares. "This has also made gamete transfer possible as an assisted fertilization technique," he says. "You can take eggs from a mare to mature *in vitro*, then transfer them to the oviduct of the recipient females and then breed those females."

Discoveries regarding the fertilization processes and changes in the egg during maturation, fertilization, and initiation of cell division are also critical to development of cloning techniques by nuclear cell transfer. As researchers keep learning more about equine conception, they are able to move forward in more ways to assist equine reproduction. —Heather Smith Thomas

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### The Conceptus

The zygote divides into two cells by 24 hours and eight cells by 72 hours, according to Tibary. Division dramatically speeds up from there. "The conceptus is ready to come down into the uterus at the late morula stage or early blastocyst stage, which is a well-developed embryo, at about 144 hours (six days from fertilization; this is why veterinarians can use intrauterine antibiotics and flush the uterus up until five days after ovulation)," he says. "Passage from the uterine tube into the uterus is directed by the embryo. In the mare, the uterotubal junction is very muscular, and only a viable embryo can open it. The embryo secretes (the hormone) prostaglandin E to stimulate the junction to relax."

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After the embryo enters the uterus it travels around, nourished by uterine fluids until it becomes fixed in one spot, usually at the tip of one of the (uterine) horns, at about Day 16 or 17. This is why vets use transrectal ultrasound to detect twins before this stage. There it attaches and grows, becoming a fetus at about Day 40 when the placenta starts to develop around it and the umbilical cord is formed.

### Take-Home Message

If every physiological detail falls into place, the paths of the egg and one sperm will cross, unite, and form a viable embryo. If not, the owner and vet might try to re-breed, hoping the timing is right and the stars will align next time. ♣

### ABOUT THE AUTHOR

Heather Smith Thomas ranches with her husband near Salmon, Idaho, raising cattle and a few horses. She has raised and trained horses for 50 years, and she has been writing freelance articles and books nearly that long, publishing 20 books (including *Care and Management of Horses*) and more than 9,000 articles for horse and livestock publications.