Embryo Transfer That Works:
Embryo Transfer As A Tool For Improving Fertility During Heat Stress

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Heat Stress and Fertility - A Big Problem!

Increases in milk yield have made cows more susceptible to heat stress than they were 10 or 20 years ago. A high-producing cow starts to experience an increase in body temperature at air temperatures as low as 80EF or less. As a result, heat stress is a problem throughout most of the country, even in places as far north as Wisconsin and New York. Cows that are exposed to heat stress suffer decreased feed intake, reduced milk yield, poor expression of the signs of estrus, and infertility.

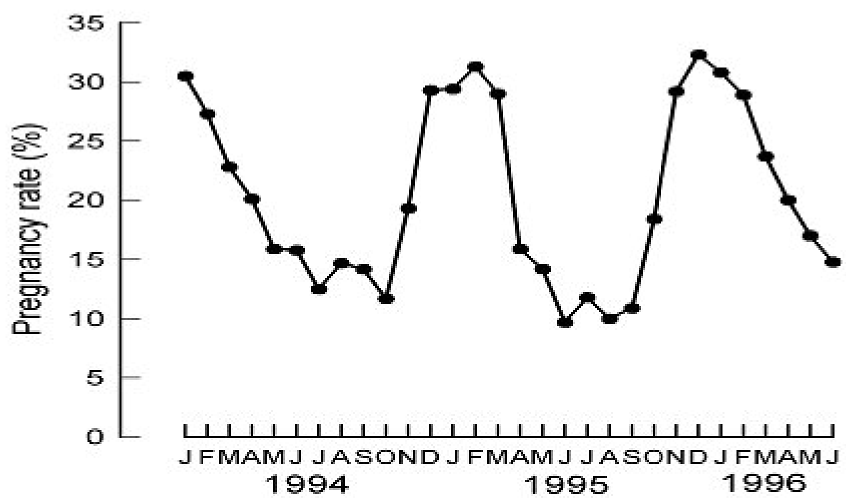

Figure 1. Seasonal variation in pregnancy rate (number pregnant/number inseminated) on a commercial dairy in Florida in which cows were housed in facilities with shade, fans and sprinklers. Data are from Hansen and Aréchiga, J. Anim. Sci. 77 (Suppl 2): 36-50 (1999).

Fertility can be measured several ways - one good indicator is pregnancy rate per AI (also called conception rate). Pregnancy rate per AI is the proportion of cows that get pregnant following insemination. In Florida, heat stress has a large negative effect on pregnancy rate per AI even when cows are housed in structures designed to keep cows cool. The data in Figure 1 illustrate this phenomenon. These data are taken from a commercial dairy in Okeechobee County, Florida, where cows were housed in well-ventilated barns with shade, sprinklers and fans. Despite these efforts to cool cows, the proportion of cows that became pregnant after insemination dropped below 15% in the summer months. What this result means is that, in many cases, we cannot depend solely on efforts to increase cow comfort if we want to keep herd fertility high during the summer.

Why Embryo Transfer?

Infertility in the summer is the result of disruption in the events in the cow leading to a healthy embryo. Heat stress acts primarily at two phases of the cow's reproductive process. First, heat stress disrupts the normal processes in the follicle - the structure where development of the egg (or oocyte) takes place. As a result, heat-stressed cows tend to produce oocytes that are not capable of becoming normal embryos after fertilization. Secondly, cows exposed to heat stress typically have elevated body temperatures that are high enough to block development and eventually kill the embryo. Even if an oocyte from a heat-stressed cow has the potential to develop into a normal embryo, it is likely that the embryo will not be able to develop properly.

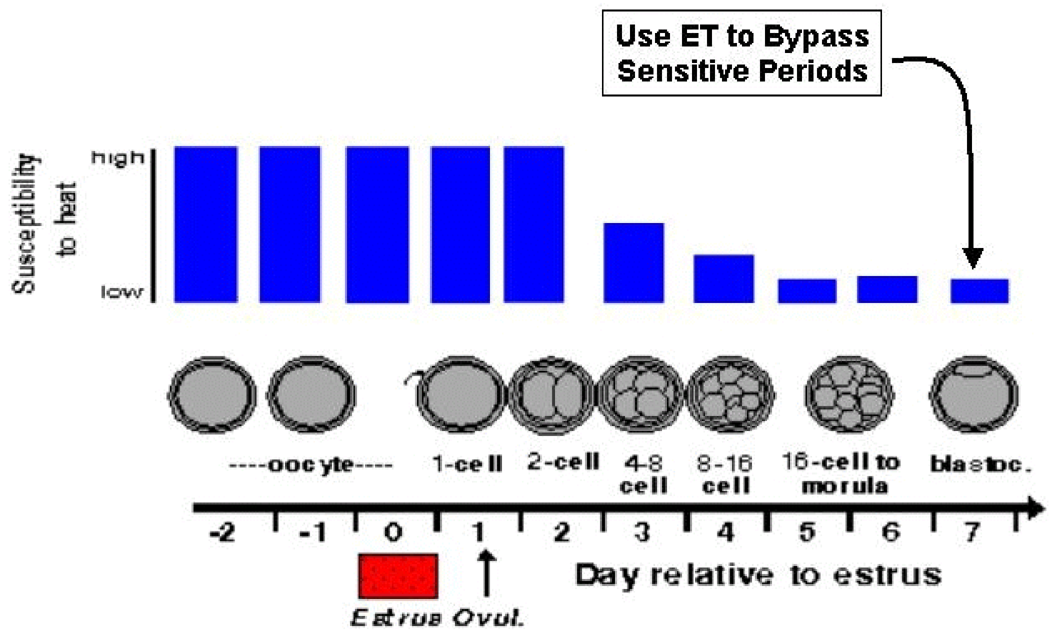

Figure 2. Illustration of mechanism by which embryo transfer can be used to increase fertility in heat-stressed cows. Before estrus, the oocyte growing within the follicle is very sensitive to disruption by exposure of cows to heat stress. After estrus and ovulation, the oocyte and newly fertilized embryo is also very sensitive to disruption by heat stress. As the embryo grows, though, it becomes more resistant to heat stress. Transfer of an embryo into the uterus at day 7 after estrus thus bypasses effects of heat stress on the oocyte and early embryo.

One of the features of embryonic development is that embryos become more resistant to various stresses as they become older. Thus, heat stress on the day after breeding can block embryos from developing while heat stress at seven days after breeding has little effect on embryonic survival. The use of embryo transfer to improve fertility in the summer is based on the fact that embryos are typically transferred into the uterus of recipient cows at day 7-8 after estrus, a time when the embryo has already passed the period when it is most susceptible to elevated temperature (see Figure 2). Effects of heat stress on the oocyte are avoided because the only embryos transferred are those derived from oocytes with sufficient quality to give rise to transferable embryos. Embryos can also be produced during cool months of the year, when the oocyte is not susceptible to heat damage, and then frozen until transfer in the summer.

Does Embryo Transfer Work?

When fertility to AI is low because of heat stress, embryo transfer can result in higher pregnancy rates, provided embryos are of sufficiently high quality. Probably the best embryos that can be transferred in terms of chances of establishing pregnancies are embryos produced by superovulation. In this procedure, donor cows are treated with multiple injections of follicle-stimulating hormone to cause growth and ovulation of multiple follicles. Embryos are flushed from the uterus by use of a Foley catheter at 7-8 days after breeding and embryos at the correct developmental stage are either transferred immediately into recipients or frozen for transfer at a later time. For optimal results, recipients should be at the same stage of the estrous cycle (± 1 day) as the donor cows at the time of flushing. As shown in Figure 3, transfer of fresh (i.e., non-frozen) embryos from superovulated cows into heat-stressed recipients improved pregnancy rates from 13.5% for AI to 29.2% for embryo transfer. An increase in pregnancy rate using superovulated embryos is also seen when superovulated embryos are transferred after long-term storage by freezing. In particular, it was found that pregnancy rate for AI was 24.1% vs 35.4% for cows receiving a frozen-thawed superovulated embryo.

Superovulation is a time consuming and expensive procedure. Generally, 4-8 transferrable embryos are produced per superovulation procedure at a cost of about $75-125 per embryo. Donor cows can be used no more frequently than once a month and, more commonly, less often.

Another method for producing embryos is through in vitro fertilization (IVF). In this procedure, oocytes recovered from donor cows are fertilized in the laboratory. Oocytes can be recovered from living donors using a procedure called transvaginal, ultrasound-guided, oocyte recovery (or oocyte pickup or OPU for short). One advantage of OPU over superovulation is that the procedure can be produced as often as twice weekly and can be performed on pregnant cows as well as open cows. Oocyte pickup is an expensive procedure, however. A typical fee for a single collection (yielding 1-2 transferrable embryos) is $400. Moreover, OPU requires highly skilled personnel and specialized equipment. OPU is best used when the goal is to obtain

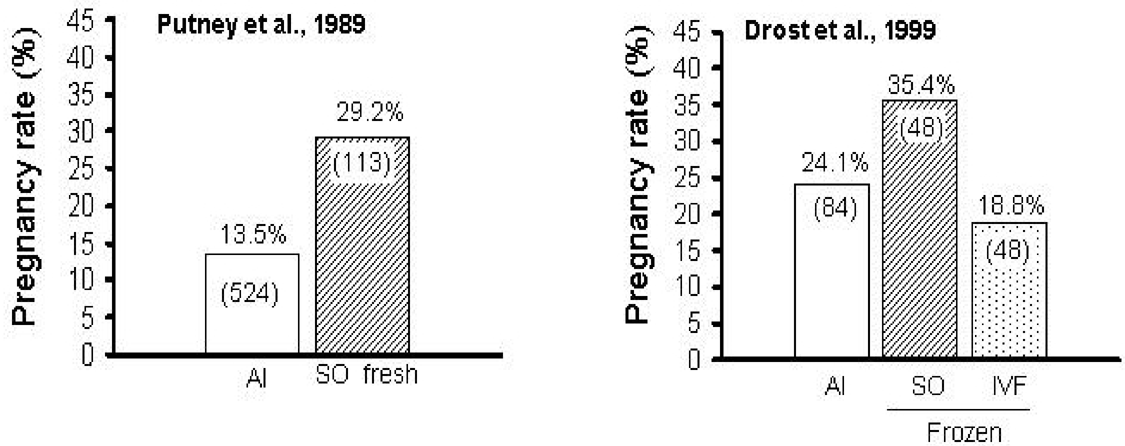

Figure 3. Effectiveness of embryo transfer for improving pregnancy rates of lactating dairy cows during heat stress. Experiments were conducted in Florida during the hot period of the year. Embryos were transferred after collection (fresh) or after cryopreservation (frozen). Pregnancy was determined by rectal palpation at 40-60 days of gestation. Abbreviations are AI=artificial insemination, SO=superovulation, and IVF=embryo produced by in vitro fertilization. Numbers above each bar represent percent pregnant and numbers in parentheses are the number of recipients. Data are from Putney et al. (Theriogenology 31: 765-778 (1989)] and Drost et al. [Theriogenology 52: 1161-1167 (1999)].

offspring from valuable donor cows.

An alternative IVF procedure is to harvest oocytes from ovaries collected at a slaughterhouse. While the identity of the donor animal is not known, the cost of producing embryos from slaughterhouse oocytes is relatively cheap. Estimates for commercial production of embryos using slaughterhouse oocytes is about $15 to $30 per embryo. Among the companies that will produce embryos using slaughterhouse oocytes are BOMED (Madison, Wisconsin) and Transova (Sioux Center, Iowa)

The genetic value of embryos produced by slaughterhouse embryos can be high. It has been shown that the predicted transmitting ability for milk yield for cows sent to slaughter is only slightly less than the overall population of cows. Moreover, since one straw of semen can be used in IVF to produce dozens of embryos, embryos of high genetic potential can be produced inexpensively. When sexed semen comes on the market, its use can be incorporated into IVF systems to produce large numbers of female embryos inexpensively.

Like for superovulated embryos, pregnancy rates in the summer can be improved by transferring embryos produced in vitro to cows (see Figure 3). However, in vitro produced embryos do not survive freezing as well as embryos produced by superovulation and the increase in pregnancy rate achieved by transfer of embryos produced in vitro is only seen when fresh embryos are transferred (Figures 3-4). Pregnancy rates achieved following transfer of a frozen-thawed embryo produced in vitro were no higher than what is achieved by AI.

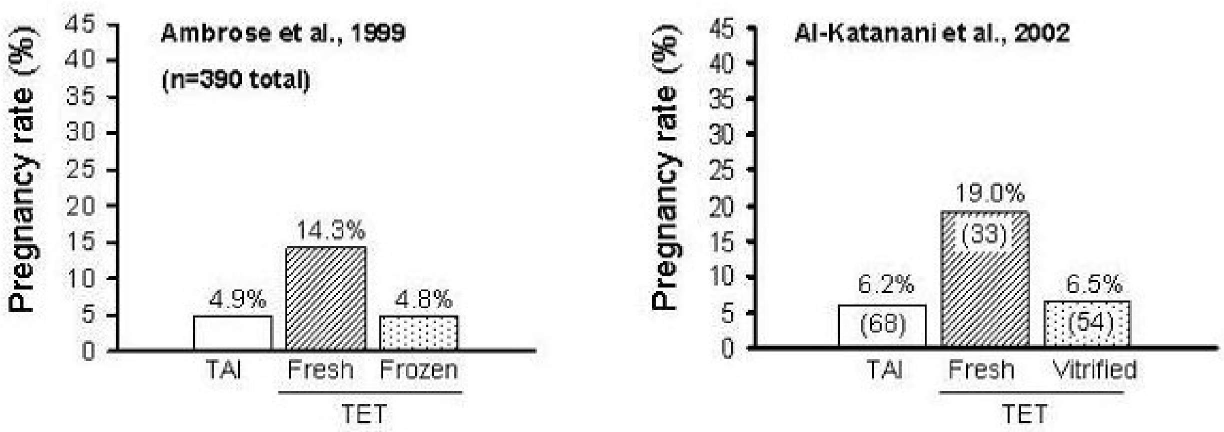

Figure 4. Effectiveness of timed embryo transfer using embryos produced in vitro for improving pregnancy rates of lactating dairy cows during heat stress. Experiments were conducted in Florida during the hot period of the year. Ovulation was synchronized using the OvSynch procedure and cows were either inseminated at a fixed time (timed AI; TAI) or received an embryo at a fixed time (timed embryo transfer; TET). Embryos were either unfrozen (fresh), or were frozen by conventional procedures (frozen) or by vitrification (vitrified). Pregnancy was determined by rectal palpation at 40-60 days of gestation. Numbers above each bar represent percent pregnant and numbers in parentheses are the number of recipients. Data are from Ambrose et al. [J. Dairy Sci. 82: 2369-2376 (1999)] and Al-Katanani et al. [Theriogenology 58: 171-182 (2002)].

Getting Around the Problem of Estrus Detection

Use of embryo transfer in the summer is made difficult by the fact that estrus detection is reduced during heat stress. It is inefficient to place embryos into recipient cows at 7 or 8 days after estrus when estrus is not observed in 50% or so of the cows. Fortunately, the same hormonal treatments that can be used to synchronize ovulation for AI can also be used to synchronize ovulation for embryo transfer. In our hands, we have transferred embryos into recipients in which ovulation was synchronized with the GnRH-PGF-GnRH scheme used for OvSynch (with or without prostaglandin presynchronization). Pregnancy rates achieved with timed embryo transfer (TET) are shown in Figure 4 and a typical timed embryo transfer scheme is detailed in Table 1.

Table 1. Typical schedule for timed embryo transfer.

Day	Activity
Day 0	Inject 100 µg GnRH (2 ml), i.m. (Cystorelin®, Factrel®, or Fertagyl®)
Day 7	Inject 25 mg prostaglandin F-2" (5 ml), i.m. (Lutalyse®)
Day 9	Inject 100 µg GnRH (2 ml), i.m.
Day 10	(Day of ovulation)
Day 17-18	Transfer either fresh superovulated embryo (cow mated on Day 10), frozen superovulated embryo, or fresh in vitro produced embryo fertilized on Day 10 Transfer should be performed only on cows with a corpus luteum - place embryo in the uterine horn adjacent to the corpus luteum

Other Problems with Embryos Produced In Vitro

The inexpensive cost of embryos produced using slaughterhouse oocytes make them a compelling choice for embryo transfer schemes whose primary purpose is to increase fertility during summer. It is important to remember, however, that there are additional problems associated with the use of these embryos. In addition to not freezing well, a high rate of fetal loss has been reported following transfer of embryos produced in vitro. In our hands [Block et al., J. Anim. Sci. 81:1590-1603 (2002)], 24% of cows pregnant at day 53 of pregnancy lost their pregnancy before calving. This compares to a value of about 10% for lactating cows bred via AI. There can also be a higher incidence of prenatal mortality associated with calves produced by in vitro fertilization (Figure 5). Calves produced from in vitro produced embryos also tend to be larger at birth - in our system, Holstein calves produced from in vitro-produced embryos weighed an average of 94 lb at birth. Occasionally, very large calves (as much as 200 lb) can be born. The occurrence of these grossly oversized calves is less frequent than was previously the case because of changes in culture conditions (removal of serum from the growth medium used to produce embryos).

In many culture systems used to produce embryos, a greater proportion of transferrable embryos are male. In our last study [Block et al., J. Anim. Sci. 81:1590-1603 (2002)], 64% of calves born from embryo transfer using embryos produced in vitro were male. In the future, changes in culture conditions should eliminate this problem. Indeed, sexed semen, which has now been developed and will one day become commercially available, is ideally suited for IVF since one straw of semen can be used to produce multiple embryos.

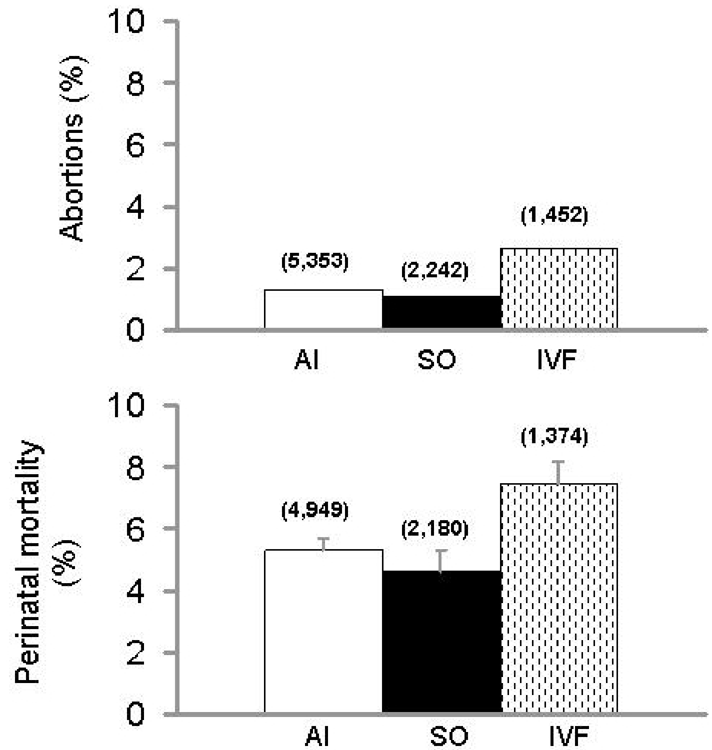

Figure 5. Rate of abortions and perinatal mortality from a large dataset of calves produced in the Netherlands. The number above each bar represents the number of calves. Data are from van Wagtendonk-de Leeuw et al., Theriogenology 49: 883-894 (1998).

Where We Are Now

Clearly, much work needs to be done to make embryo transfer a reproductive management tool for heat-stressed cows that can be easily implemented on the farm. It is possible, however, to implement an effective embryo transfer program in the summer with existing technology. Superovulated embryos (either fresh or frozen) and fresh, in vitro-produced embryos will both work in such a program. Superovulated embryos are expensive and, as a practical matter, can be in short supply for large dairies. In vitro-produced embryos of high genetic merit can be produced inexpensively in unlimited quantity by the use of slaughterhouse oocytes and fertilization with semen from bulls of high genetic merit. Incorporation of oocyte pickup technology allows further opportunities for genetic improvement (since oocytes can be harvested from genetically-valuable females) but also increases the costs and labor involved. Regardless of the source of oocytes for in vitro fertilization, the need to transfer embryos fresh means that the recipients need to be subjected to ovulation synchronization (a good idea in any case) and that synchronization of cows be coordinated with the company producing the embryos.

Making in vitro produced embryos using slaughterhouse oocytes is not technically demanding but the companies that offer this service do not presently produce large numbers of embryos for commercial dairy farmers. Thus, the dairyman will have to work closely with the embryo production facility to ensure that the required type of embryo is available for his needs. Personal discussions with people in the embryo production industry indicate their willingness to do so.

The actual transfer of embryos can be done by a skilled inseminator. However, transfer of embryos is not quite as simple as performing AI since the technician must be able to identify the corpus luteum so as to place the embryo in the uterine horn on the side of the corpus luteum. Failure to do so will lead to failure of the pregnancy. Care must also be taken to avoid contamination of the uterus with microorganisms - special sheaths are available to cover insemination rods to avoid dragging materials into the uterus.

Where We Are Going

Much research is being conducted to make embryo transfer a simpler procedure to carry out. In large part, this means developing procedures to freeze in vitro produced embryos, either by changing the conditions used to culture embryos or the procedures used for freezing. The solutions used to freeze embryos currently are often toxic and embryos need to be washed in a petri dish before transfer. One goal is for direct transfer of embryos where a straw containing an embryo is thawed and loaded into a transfer pipette for placement in a cow without the need for the technically-demanding washing steps. Vitrification is one freezing procedure that offers promise. In this procedure, high concentrations of freezing reagents are used and the embryo freezing medium is transformed into a glass-like state instead of ice.

Research is also being carried out to improve the pregnancy rate that can be achieved by embryo transfer. Experiments are underway to improve embryo culture conditions, identify markers on the embryo that would allow transfer of only those embryos that have a high probability of surviving freezing, and developing hormonal treatments of recipients that will increase pregnancy rate.

Sexed semen is now technically possible and companies exist in the United States, Argentina, and England that produce such semen. Given the high costs of such semen, embryo transfer is a cost-effective approach for utilizing sexed semen since one straw of semen can be used to inseminate dozens of oocytes. Unfortunately, sexed semen is not yet marketed in the United States.

Take-Home Message

Infertility in the summer can seem like an unsolvable problem. Cooling cows certainly helps but fertility still can drop dramatically in the summer. Problems of poor estrus detection during hot weather can be eliminated by use of timed AI but the fertility to these inseminations can be very low. Provided one can obtain an inexpensive supply of embryos, and has personnel with the skill to transfer embryos, use of embryo transfer represents the best approach for increasing pregnancy rate during heat stress.

The best embryos in terms of pregnancy rate and minimal abnormalities are those produced by superovulation. These, however, are expensive and in short supply. A useful alternative is to produce embryos in vitro using slaughterhouse ovaries. These embryos can be produced very inexpensively and, by using sires with high genetic merit, genetically-valuable embryos can be obtained. Because they freeze so poorly, in vitro produced embryos should be transferred without freezing and this requirement will necessitate use of ovulation synchronization protocols such as the OvSynch program. Transfer of in vitro produced embryos is associated with increased rates of fetal and neonatal loss but the risks of these problems will in many cases be less than the benefits of bypassing effects of heat stress on fertility.

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