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EVOLUTION OF EMBRYO TRANSFER IN DOMESTIC ANIMALS

Evolución de la transferencia de embriones en animales domésticos

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INTRODUCCIÓN

Reproductive biologists have been fascinated by the idea of embryo transfer (ET) in mammalians since the early work of Heape in rabbits in 1890. The development of this technology was initially slow but there have been great advances in the latter part of the 20th century as elegantly reviewed by Betteridge.[16] The first successful transfers (marked by birth of offspring) were reported in small ruminants and bovine species in 1930's and 50's, respectively. The first successful embryo transfer in the equine was reported in 1972.[56] The first significant advances in camelid ET occurred in the mid 80's and early 90's.[9,115] ET became a popular reproductive biotechnology in the late 1970's and early 1980's. Commercial use became commonplace first in cattle.[134] Today most bulls used for artificial insemination are produced by ET. The technique became widely used by producers shortly thereafter in other species such as equine, sheep, and goats, and more recently camelids and domestic small animals. The main advantages of ET are to capitalize on the genetic potential of females, reduce the generation interval, reduce the risk of disease transmission, and reduce the cost on international trade of genetics (avoid transportation of live animals). In performance animals (equine, racing camels), the use of ET allows reproduction of valuable females while they are in training or performance and avoid risks associated with pregnancy and parturition. In addition, advances in in vitro production and manipulation of embryos allowed for more rapid multiplication of superior females and males and use of gametes from infertile, terminally ill, or dying females.[50,113] The potential for increased return from ET has also benefited from other biotechnologies such as the use of sexed semen and optimization of superovulation and embryo cryopreservation systems.[29,45,91] The objective of the present paper is to give an overview on embryo transfer technology in domestic animal species.

SUPEROVULATION

The most critical aspect in optimization of embryo transfer as a service to producers is the development of reliable multiple ovulation and embryo transfer (MOET) system through hormonal ovarian superstimulation.[92] Several techniques have been devised to stimulate follicular recruitment and multiple ovulations. These are based on administration of follicle stimulating hormone (FSH) or other hormones with FSH activity such equine chorionic gonadotropin (eCG) or human menopausal gonadotropin (hMG). FSH from porcine origin (pFSH) has been the most widely used formulation in a variety of ruminant species and camelids.[20,31,84,115] In the equine, pFSH is less efficient for superovulation. FSH of ovine origin (oFSH) has been used primarily in ruminants.[92] More recently purified equine FSH (eFSH) and recombinant equine (reFSH) produced more reliable ovarian stimulation in mares.[7,10,65,85,87,101]

Equine chorionic gonadotropin is widely used for ovarian superstimulation either alone or in combination with pFSH primarily in ruminants and camelids;[9,32,51,84,115,117] however, the use of this hormone has been progressively abandoned because of female refractoriness to the treatment and the large variability in obtained results. These problems are associated with its significant LH activity and longer half-life.[9]

As an alternative to ovarian stimulation with exogenous hormones, active or passive immunization against inhibin has been attempted in several species with variable results.[67,79,112,130]

Ovarian response to FSH is highly dependent on the dose and timing of injections in relationship to the normal follicular wave dynamics. FSH is administered twice daily either at constant or decreasing doses over 3 to 5 days in ruminants[63] and camelids[66,84,115] or at constant dose in equine until development of follicles which are 30 or 35 mm diameter.[65] Studies over the last 2 decades showed that it is essential that FSH treatment is initiated before follicular deviation and dominance.[52,63] The dominant follicle may be eliminated by induction of ovulation or ablation (transvaginal ultrasound guided aspiration).[55] An alternative to aspiration to eliminate dominant follicles is the administration progesterone and estradiol.[18,19,23,63,84,87] In the equine and camelid, the best results are obtained when FSH treatment is initiated a few days after ovulation when the ovaries display only cohorts of very small follicles (<25 mm in mares and < 4 mm in camelids) (Figure 1).[9,65,87,117]

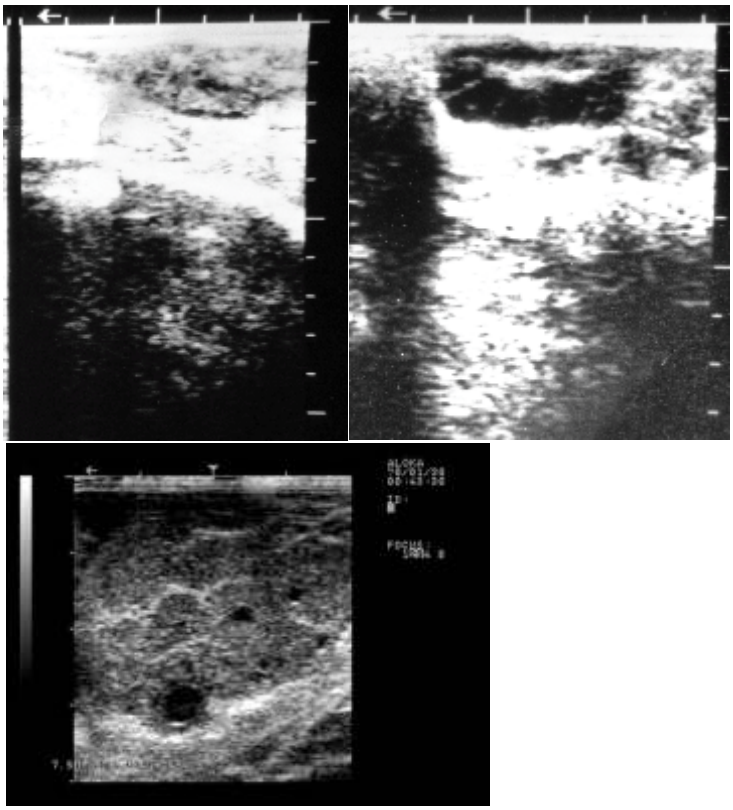

Figure 1. Ultrasonography of the camel ovary. a) at initiation of FSH treatment for superovulation , b) 2 days before mating note the uniform follicular response. c) on the day of embryo collection note the corpora lutea.

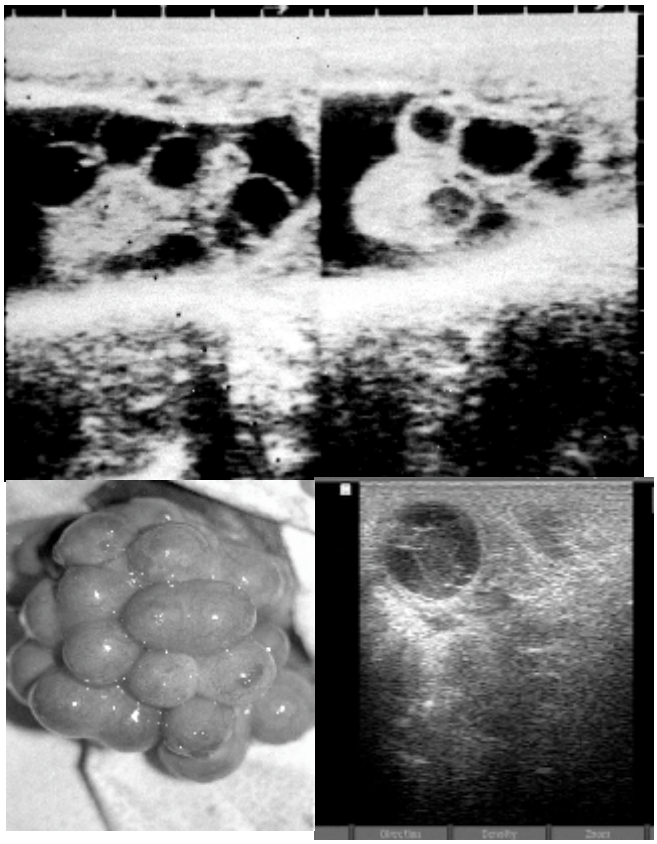

Figure 2: Abnormal superovulatory response in camels. a) premature luteinization, b) large hemorrhagic follicle, c) overstimulated ovary exposed surgically.

Recombinant equine FSH has also been used successfully to stimulate and produce embryos from seasonally anestrous mares.[69,70,87,88] Superovulation in the mare is however limited in terms of number of recovered embryos (average 1.9) likely due to anatomical limitations requiring ovulation to occur through the ovulation fossa.[102]

There is wide animal to animal variation despite attempts at optimizing response to FSH treatment. Abnormal responses include lack of follicular development, follicular cysts, premature luteinization of excessive response (overstimulation) (Figure 2). Recent studies showed that this variation may be explained by breed, age, genetics, nutrition (body condition score, trace minerals), and stress (environmental or handling).[128] [74,75,129] Polymorphisms of the bovine growth differentiation factor 9 gene have been found recently to be associated with superovulation performance in Holstein cows.[109] Recently, it was shown that circulating anti-mullerian hormone (AMH) levels during the first lactation is highly correlated with superstimulation response and embryo production in cows.[75]

Superovulation regimens requiring multiple injections of FSH are deemed impractical and stressful to embryo donor animals, particularly in ruminants. Alternatives using a single subcutaneous injection of the total dose of FSH showed good results if administered in an area with sufficient adipose tissue. A more recent approach uses a slow release polymer (hyaluronan) as a carrier for FSH. This preparation produces adequate superovulation with two intramuscular injections 48 hours apart.[20,44,63,121]

Effect of superstimulation on embryo quality has been debated over the years. There are no effects on gene expression related to ovulatory capacity, oocyte competence and embryo development in cattle;[15,26] however, overstimulation often results in poor fertilization and poor or no embryo recovery in camelids.[9,115]

Approaches to superovulation in sheep [8,64,68] and goats [13,68,116] are similar to those used in the bovine. The main techniques used to control follicular dynamics prior to superovulation are progestogen vaginal pessaries or induction of ovulation (day 0 protocol).[116] Progesterone supplementation after mating has been recommended by some authors because superovulated goats have the tendency to undergo premature corpus luteum regression resulting in poor embryo development and recovery.[116] It is also recommended to limit the number of superovulatory treatments per season in goats to three.[57]

EMBRYO COLLECTION AND HANDLING

Embryo collection methods

Non-surgical collection of embryos from the uterus is the most widely used technique in bovine, camelid and equine. Techniques (2-way Foley catheter) developed for the bovine have been adapted with excellent success for camelids.[9,104,127] The preferred technique in cattle is to flush each horn separately, while in equine and camelids both uterine horns are flushed at the same time by placing the Foley catheter balloon just cranial to the internal cervical os. Cervical catheterization in alpacas may be challenging because transrectal palpation is not always possible.[104] In these cases, catheterization of the cervix may be attempted via a speculum using a smaller (Fr. 5) Foley catheter. Sedation and epidural analgesia are often required in camelids.[127]

Non-surgical embryo flushing from the uterus is performed on day 7 after artificial insemination in the bovine and day 7 to 8 after ovulation in the equine.[102] In camelids, the literature on the best timing for embryo recovery from the uterus remains unclear because some authors report the timing based on mating while others report timing based on ovulation. The most common timing for flushing is day 8 to 9 after mating. Camelids are induced ovulators with ovulation occurring between 26 to 48 hours after mating. There may be some effect of superovulation on ovulation timing and embryo development.[9,81,98,104,127]

Embryo collection rate in the bovine is variable due to the use of superovulation but in general 5 to 8 embryos are collected per donor. In camelids and equine, where embryo collection is often performed without superovulation recovery rates can reach up to 90% in well managed fertile donors.[9,61,102,114,127] Recovery rates per ovulation are lower and extremely variable in superovulated camelids.[112] In the equine, factors that lower the embryo recovery rates significantly are use of frozen-thawed semen, advanced age of the donor and timing of flushing (day 6 instead of days 7-8).[102]

In small ruminants, collection of embryos from the uterus is performed on day 6 to 7 after mating or insemination. Surgical embryo collection following exposure of the uterus via a midline celiotomy is the most commonly used approach and yields the highest embryo recovery rates.[8,58] In recent years, laparoscopic assisted techniques have gained a lot of popularity because of the safety for the donor. However recovery rates are generally 15% lower than with surgical collection.[116] Non-surgical uterine flushing with acceptable embryo recovery rates have been reported in large goat breeds, but catheterization of the cervix is possible in only 60% of the females.[31,58]

There is little data on early embryo development and descent into the uterus in the canine and feline species. Reported timing of embryo descent into the uterus varies from 8.5 to 10 days in bitches.[1,30,124] This probably reflects differences in timing ovulation and fertilization and probably differences amongst breeds. In one study on Labradors, 16-cell morula migrated into the uterus by day 10 post-LH surge and developed to the blastocyst stage by day 12 to 13 post-LH surge.[107]

Flushing media used for embryo recovery varies from simple lactated Ringer's solution to variations of Dulbecco's phosphate buffered saline. Today most operations use commercially available flushing media. Addition of fetal calf serum to the flushing medium was progressively replaced by use of complete media containing surfactant. When a large volume of flushing medium is used (bovine, camelid and equine), fluid is directly recuperated through a commercial embryo filter that serves also as an embryo searching dish.

Embryo washing and evaluation

Embryo handling should be performed in a clean area which is protected from temperature changes, direct sunlight, and dust. Ideally, the dissecting microscope should be placed under a laminar flow hood. Embryos should be immediately transferred into small dishes containing a holding medium and evaluated for quality. Several systems for the evaluation of the quality of embryos have been proposed for various species (see International Embryo Transfer Society guidelines).[9,102] These are primarily based on presence of fertilization, stages of development as compared to stage of collection, integrity of the zona pellucida, shape of the embryo, number and morphology of the blastomeres, presence of degenerative changes, and degree of expansion. More advanced techniques for the evaluation of embryo viability are based on specific staining techniques but these remain mostly a research tool.[102] Embryo grade affects pregnancy rate and determines suitability for cryopreservation. Embryos should be washed according to IETS requirements before transfer.

Most embryos collected from ruminants are in the morula or blastocyst stage. Equine embryos are generally collected at the expanded blastocyst stage (day 7 to 8) (Figure 3), unless destined for freezing (day 6 to 6.5). In camelids, the chronology of embryo development remains relatively poorly studied.[9,81,115] Embryos are collected at the hatched blastocyst stage. Embryos from superovulated camelid females show a large variation in size (Figure 4).

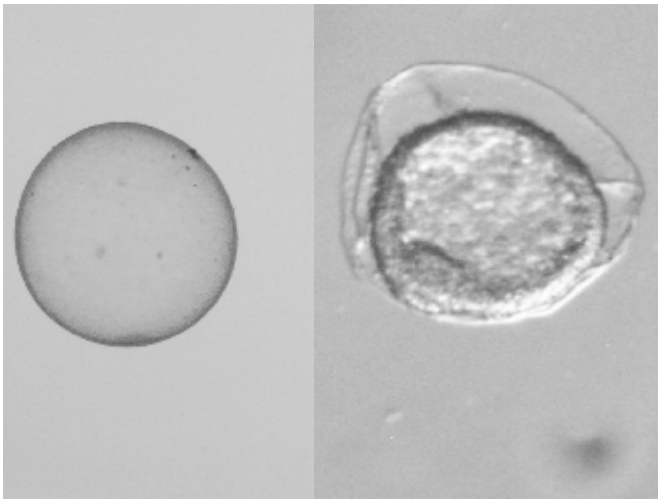

Figure 3: Equine embryos. Day 8 blastocyst with capsule, Day 8.5 expanded blastocys

In the canine species, embryos can be collected either surgically or non-surgically. Embryos are in the morula stage at 8 days after ovulation and blastocyst by day 10.[124] In cats, expanded blastocysts are recovered surgically on day 6 to 8 after mating and induction of ovulation with hCG.[90,123]

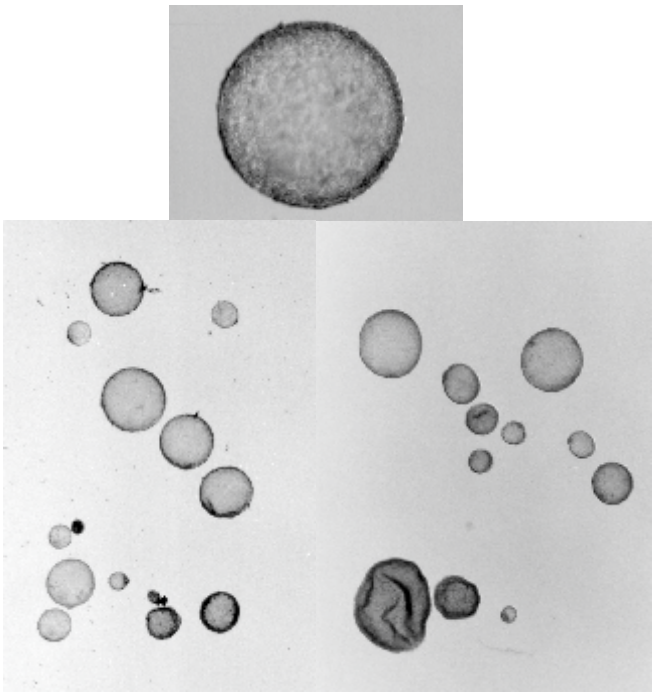

Figure 4: Camel embryos. Hatched blastocyst collected on day 8 post-mating, b) and c) group of embryos collected fro superovulated donors on day 8 post-mating. Note de large variation in size amongst embryos

Embryo manipulation

Several types of embryo manipulations have been studied over the years aiming mainly at production of twins (embryo splitting) or determination of the sex of the embryo.[34,41,82,83,112] More recently embryo biopsy has been used for genetic testing.[49] It is important to note transfer of a single embryo has resulted in monozygotic twin pregnancy in the mare suggesting that ex utero manipulation may result in spontaneous embryo splitting.[62]

Factors affecting success rate after transfer

Under ideal conditions pregnancy rates produced by ET vary between 50 to 85% according to species.[42,114] In addition to operator experience, embryo quality, recipient selection, and management are the most critical factors for the success of any embryo transfer program. Large sets of field data have been accumulated over the years, particularly in the bovine and the equine showing recipient effect. In the bovine, the most important factors are age, lactation and nutritional status.[42,54,60,80] Similar effects of lactation, age, health and body condition have been reported in camelids.[9,104,127]

The degree of synchrony between the donor and recipients has been studied in all species and results indicate that the ideal recipient should ovulate synchronously or up to one day after the donor. In the equine, recipients that have ovulated up to 3 days after the donor may be utilized for embryo transfer.[102] Anestrous and ovariectomized hormone-treated mares have been utilized successfully as long as they receive progesterone or altrenogest for the first 100 to 120 days of pregnancy.[38,114] Mares that have ovulated up to 6 days before the donors have been utilized under certain conditions. The use of meclofenamic acid to eliminate PGF2α release was used in mares and camels to maintain CL function in asynchronous recipients.[95,131,132] In camels, progesterone-treated females may be used as recipients however treatment should continue throughout pregnancy. This practice is discouraged because of the high risk of complications at parturition.[115] Instead, treatment with eCG after transfer of embryos has been used to create accessory CL's and help to maintain pregnancy in camel recipients.[115]

Although surgical embryo transfer was shown to produce better pregnancy rates, today almost all ET in bovine, equine and camelids is performed non-surgically. Non-surgical techniques have been utilized in goats but the majority of transfers in small ruminants are performed surgically (laparotomy or laparoscopy). In equine and camelids, catheterization of the cervix was believed to induce release of PGF2α and cause premature luteolysis in recipients but recent studies have shown that this is not the case.[102,118] In the bovine, treatment with flunixin meglumine seems to improve pregnancy rates in recipients with a hard to catheterize cervix.[86]

Embryos are transferred ipsilateral to the corpus luteum (CL) bearing ovary in cattle. In mares, the embryo may be deposited in the uterine body. In camelids, the effect of side of transfer in relationship to CL location remains somewhat debated but data suggest that ipsilateral side produces superior pregnancy rates.[9,127] In sheep and goats at least two embryos are transferred per recipient. Single or multiple embryos are transferred surgically into the uterus of canine and feline species but there is very little data on success rates.[30,123-125]

Embryo preservation

Embryo preservation adds a powerful dimension to the use of ET in practice. The need to maintain synchronized recipients on site can be eliminated. Short term preservation (24 hours) through cooling has been utilized primarily in the equine. Embryos have been traditionally packaged in Ham's F10 medium and slowly cooled to 5°C and shipped in an Equitainer®. The original medium was prepared by passing a mixture of 5% CO_2, 5% O_2 and 80% N_2 through the medium to correct the pH. More recently other commercial synthetic embryo holding media have been utilized for cooling (Emcare®, Vigro holding plus®).[22,28,73,76-78]

Long term preservation by cryopreservation is a more attractive technique. Two techniques have been used: slow freezing and vitrification. Slow freezing is based on a controlled rate of cooling followed by seeding when a desired temperature (-5 to -7°C) is reached, then further cooling until -35°C at a rate of 0.5°C/minute before plunging into liquid nitrogen.[43,122] This system requires stepwise addition of the cryoprotectant (glycerol, DMSO, 1,2 propenediol, ethylene glycol) during the preparation of the embryos. The cryoprotectant is removed stepwise or in a single step by passage into a high concentration sucrose solution. Embryo cryopreservation using this method has been efficacious in cattle, sheep, and goats with little to no loss in pregnancy rates compared to fresh embryos when good quality embryos are used. However, the slow freezing method produced poor results in camelids and equine.[66,103,115] There are several morphological (larger size, absence of zona pellucida, presence of a capsule in equine) and biochemical reasons (toxicity of glycerol, high lipid content and difference in lipid composition) that contribute to failure of cryopreservation of embryos by the slow freezing method.[33,94,103] Large embryos are more prone to sustain cryoinjury due to ice crystal formation[92] and disruption of the cytoskeleton.[97]

Vitrification methods for embryo cryopreservation were developed to circumvent the effect of slow freezing (ice formation), and eliminate the computerized embryo freezing units. This process requires an ultrafast freezing rate in the presence of very high concentrations of cryoprotectant (Figure 5). Excellent results have been achieved with this technique in a variety of species.[24,35,53,103] In the equine, transfer of vitrified-thawed embryos yields good results if the embryos are less than 300 microns in diameter when frozen (collected at 6 to 6.5 days). Recent studies have shown that the combined negative effects on cryopreservation of the capsule and size of the embryo can be eliminated by mechanically collapsing the expanded blastocyst and reducing the blastocele size.[25,93] Vitrification of camelid embryos has been reported with variable success.[4,5,96,97,115,126]

Embryo transfer using in vitro produced embryos

During the last 20 years substantial advances have been achieved in a variety of species via in vitro production of embryos through in vitro fertilization (IVF), intracytoplasmic sperm injection (ICSI) and somatic cell nuclear transfer (SCNT) or cloning. Although transfer of embryos produced using these techniques does not always yield high pregnancy rates and resulting pregnancies or offspring have been plagued by some abnormalities (high pregnancy loss, abnormal placentation, large offspring syndrome, etc.) substantial progress has been made in refining in vitro maturation of oocytes and embryo culture (see reviews for the bovine,[2,89] camelid,[27,72,115,119,120] small ruminants,[113] equine,[6,47,48,50,71] and small animals[30]).

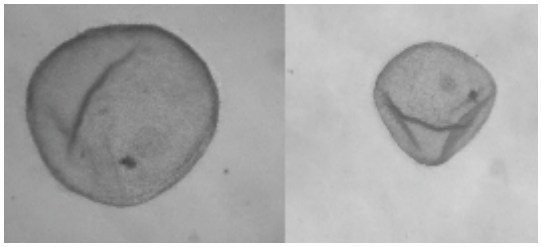

Figure 5: Embryo vitrification. Previously vitrified alpaca embryos after thawing

Conclusion

Embryo transfer using in vivo produced embryos was adopted as a field reproductive technique in most large animal domestic species. Superstimulation treatments continue to improve as our understanding of follicular dynamics and discoveries of mechanisms regulating it increase. Recombinant DNA technology will help to develop and refine hormonal manipulation of ovarian follicular dynamics not only for in vivo production of embryos but also for oocyte collection and maturation. Scientific research on ET and its associated techniques is no longer concentrated to a few geographical areas (Australia, Europe, North America, Japan) but has expanded to include several research teams in South America, Africa, Asia, and the Middle-East. Today, scientific contributions in ET and its associated techniques in the bovine, equine, and camelid species have become more global and more collaborative. This is well illustrated by the multiplication of the number of specialized international symposia and conferences in this area. Efforts to understand the welfare implications of these biotechnologies and the area of risk of disease transmission through embryo biotechnologies continue leading to development of sound production protocols and guidelines.[3,11,12,14,17,21,36,37,39,40,46,59,99,100,105,106,108,110,111,133] It is therefore very important for the practitioner or scientist involved with these biotechnologies to keep up with the scientific literature in the field.

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