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Improving Delivery and Offspring Viability of In Vitro-Produced and Cloned Sheep Embryos[1]

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ABSTRACT

Recently developed, assisted reproductive technologies (e.g., in vitro embryo production and nuclear transfer) have encountered perinatal morbidity/mortality of the offspring produced, which are likely to hinder the application of these techniques. Consequently we have sought to develop a system of hormonal stimulation that will ensure the delivery of offspring more prepared for extrauterine life. Here we examine deliveries outcome in sheep carrying in vitro-produced and nuclear transfer (NT) embryos in comparison to artificially inseminated and naturally mated control ewes. All groups (excluding NT, which received one treatment) were subjected to one of two hormonal treatments for induction of delivery, whereas the third part of each group was left without any treatment. The first (commonly used for naturally mated ewes) dexamethasone treatment did not solve a majority of parturition disturbances, and actually the number of deliveries necessitating assistance was reduced ($P < 0.05$) by this treatment in the control group. On the other hand, combined estradiol plus betamethasone stimulation (E + B) solved a majority of complications regarding delivery performance such as lack of the preparation of the mammary gland, low myometrial contractility, insufficient cervical ripening, and impaired maternal behavior. Moreover, substantial reduction of neonatal mortality was observed following the combined treatment. In conclusion, the E + B induction of delivery overcame the majority of physiological and behavioral intrapartum failures of sheep foster mothers and increased the survival of offspring, and thus can be recommended as a safe method for inducing delivery in foster mothers carrying in vitro-generated embryos.

assisted reproductive technology, behavior, embryo, estradiol, parturition

INTRODUCTION

A number of perinatal complications have been reported following the transfer of in vitro-produced (IVP) and cloned embryos to foster mothers. These complications include increases in gestational length, newborn size, incidence of parturient dystocia, and susceptibility to neonatal infections [1–7]. Although the underlying causes of these problems are unclear, evidence suggests that improving the efficiency of in vitro embryo production may alleviate many of these complications [8].

Early attempts to produce ruminant embryos in vitro used poorly defined culture systems and resulted in embryos with clear morphological differences compared to in vivo-produced counterparts [6, 9, 10]. The offspring generated by transfer of such embryos were subject to a variety of developmental defects [4, 5, 7, 11]. Since then substantial progress in terms of morphology of IVP ruminant embryos has been achieved using defined and semidefined culture systems [9, 12–14], and in recent studies there was no evidence of congenital defects, enhanced gestation length, or perinatal loss [9, 15–17]. Furthermore, a recent allometric study [18] did not replicate the earlier findings of differences in organ allometry associated with IVP fetuses [19].

However, although the problems of low efficiency and perinatal complications associated with embryo development in vitro appear to be substantively overcome, a number of problems concerning parturition still persist. In particular, these problems are most evident in instances where development to term is very low, such as with nuclear transfer procedures [20–22]. Even when the adverse effects of dystocia and preterm induced parturition are obviated by performing a cesarean section, prenatal problems persist and evidence suggests that these are due to energy metabolism defects resulting from placental insufficiency [23]. The majority of parturient complications may be explained by an inadequate signaling between mother and foetus during preparation for parturition. In some IVP and nuclear transfer (NT) pregnancies it seems that there are problems with either the maturation of the fetal hypothalamic-anterior pituitary-adrenal axis or the necessary signals required to increase ewe plasma cortisol [3]. This suggestion, based on symptoms such as a lack of preparedness for approaching parturition, a weak propulsive stage, and a physiologically immature foetus, is strengthened by evidence that experimentally restricted placental growth alters the functional development of the pituitary-adrenal axis in the sheep fetus [24]. It has also been suggested that a lower than average number of placentomes results in limited nutrient availability and gas exchange during late gestation [19], and there is evidence of impaired blood supply in placentae of animals pregnant with IVP embryos [25]. In addition, a recent study demonstrated a high incidence of developmental retardation in cloned fetuses associated with deficiencies in the establishment of placental vasculature [26]. It is possible that defects in the pituitary-adrenal axis would result in physiological and behavioral disorders of parturient foster mothers and contribute substantially to increased numbers of stillborn offspring and early postnatal loss.

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One possible approach to reducing the perinatal morbidity/mortality of offspring derived from assisted reproductive technologies is to provide the appropriate assistance during delivery. A number of procedures such as inducing parturition have been developed for this purpose; however, these protocols did not significantly reduce parturient and neonatal damage following transfer of IVP or NT embryos [2, 3, 27]. Here we report on attempts to develop procedures designed to generate assisted reproductive technology offspring more prepared for extrauterine life. In the present study we evaluate the delivery characteristics and mortality of offspring obtained by two advanced reproductive technologies (in vitro embryo production and nuclear transfer) compared to control artificially inseminated ewes. In order to completely exclude the influence of technique, we have compared our results to those obtained with naturally mated ewes. To study the effects of these hormonal treatments, pregnant recipients from all four groups were subjected to one of three delivery regimes: 1) delivery with no exogenous hormonal stimulation, 2) parturition induced by classical dexamethasone treatment (D) [3, 28] and 3) delivery stimulated by estradiol plus betamethasone (E + B) followed by an immediate postnatal injection of D to the newborn in order to ensure maturation of the lungs.

MATERIALS AND METHODS

Embryo Production In Vitro

Methods of in vitro embryo production were adapted from those previously described [29]. Briefly, following collection of sheep ovaries at slaughter, aspirated oocytes were evaluated by microscopy and only those surrounded by at least two layers of granulose cells and with evenly granulated cytoplasm were selected for in vitro maturation (IVM). Maturation medium was bicarbonate-buffered TCM-199 (275 mOsm) containing 2 mM glutamine; 10% fetal bovine serum (FBS); 5 μg/ml FSH (Ovagen, ICP, Auckland, New Zealand); 5 μg/ml LH; 1 μg/ml E; 0.3 mM sodium pyruvate; and 100 μM cysteamine. Oocytes from individual donors were incubated in 0.4 ml of medium in 4-well dishes (Nunc, Roskilde, Denmark) covered with mineral oil in a humidified atmosphere of 5% CO_2 at 39°C for 24 h. Following maturation, oocytes were partially denuded of granulosa cells by gentle pipetting in Hepes-TCM-199 containing 300 IU/ml hyaluronidase.

Fresh semen obtained from Sarda breed rams of proven fertility was used throughout the experiments. Collected ejaculates were held at room temperature for up to 2 h, centrifuged twice at 200 × *g* for 5 min, and added directly to the in vitro fertilization (IVF) medium. The IVF medium used was bicarbonate-buffered synthetic oviduct fluid enriched with 20% (v/v) heat inactivated estrus sheep serum, 2.9 mM Ca lactate, and 16 μM isoproterenol. Fertilization was carried out in 50-μl drops using 1×10^6 sperm/ml and a maximum of 15 oocytes per drop, at 39°C in a humidified atmosphere of 5% CO_2 in air, at 39°C for 20 h.

Presumptive zygotes were transferred to 20-μl drops consisting of synthetic oviduct fluid supplemented with 2% (v/v) BME-essential amino acids, 1% (v/v) MEM-nonessential amino acids, 1 mM glutamine, and 8 mg/ml BSA. Cultures were incubated in an atmosphere of 5% CO_2, 7% O_2, and 88% N_2 at 39°C with maximum humidity. At Day 3 and Day 5 of culture (Day 0 = day of fertilization), 5% charcoal-stripped FBS was added to the medium. Cultures were maintained until 7 days postfertilization, at which point embryos that developed to the blastocyst stage were transferred to synchronized ewes.

All chemicals, unless indicated, were obtained from Sigma Chemical Co. (St. Louis, MO).

Oocytes and Embryo Production In Vivo

All animal experiments were performed in accordance with DPR 27/1/1992 (Animal Protection Regulations of Italy) in conformity with European Community regulation 89/609 and in adherence with guidelines established in the Guide for Care and Use of Laboratory Animals as adopted and promulgated by the Society for the Study of Reproduction.

For nuclear transfer, oocytes were matured and embryos fertilized and allowed to develop in vivo in order to reduce extraneous influences on embryogenesis. Sarda breed ewes were maintained under field conditions at the Institute of Zootecnics Research Farm, Bonassai, Sardinia, Italy. The estrus cycles of adult ewes were synchronized during breeding season by the insertion of intravaginal sponges (40 mg FGA; Intervet, Cambridge, UK). Multiple ovulations were induced by the administration of FSH (16 mg, Ovagen, ICP) i.m. every 12 h for 48 h. Ewes were anesthetized with acepromazine maleate (0.05 mg/kg BW) and pentothal sodium (10 mg/kg BW). Metaphase II oocytes and 16-cell embryos were surgically collected 54 and 142 h, respectively, following sponge removal.

Cloning Procedures and Culture of Reconstructed Embryos

Procedures used for nuclear transfer and embryo reconstruction were those described previously [30]. Briefly, oocytes were held in TCM-199-Hepes plus 10% calf serum, 5 μg/ml Hoechst 33342, and 7.5 μg/ml cytochalasin B for 15 min. The metaphase plate was localized by short UV exposure and the chromosomes aspirated along with a small portion of cytoplasm using a micropipette. Following enucleation, oocytes were placed in a fusion chamber filled with a solution of 0.3 M mannitol, 0.100 mM $MgSO_4$, and 0.050 mM $CaCl_2$, and a pulse of 1.25 kV/cm was applied to the chamber for 80 μsec. Cytoplasts were then cultured in Hepes-TCM-199 plus 10% calf serum at 39°C in an atmosphere of 5% CO_2 until reconstruction. Pronuclear formation was used to determine activation rate by Hoechst staining of aspirated karyoplasts 8–10 h after the application of an identical electric pulse.

Zonae pellucida were removed using a glass needle, and embryos were exposed to Ca^{2+} Mg^{2+} free PBS containing 7.5 μg cytochalasin B and 2 μg nocodazole per milliliter for 20 min to facilitate blastomeres separation. Embryo reconstruction was accomplished 4 h postactivation by transferring the blastomere into the perivitelline space of the enucleated oocyte. The fusion partners were manually oriented in the fusion chamber and subjected to 1.25 kV/cm for 80 μsec in isotonic mannitol without calcium ions.

Reconstructed embryos were cultured in a humidified atmosphere in Hepes-TCM-199 plus 10% calf serum and 7.5 μg/ml cytochalasin B at 39°C for 1 h, checked for fusion, and then embedded in agar and transferred into the oviduct of a synchronous ewe. A glass filter was secured to the ampullary region of each oviduct in order to avoid embryo losses via the fimbria [31]. After 6 days the filters were removed and the agar chips collected by retrograde flushing of the oviduct. Embryos were dissected from the agar and scored for development according to morphology.

Embryo Transfer

All in vitro-produced and reconstructed in vivo-cultured embryos that developed to the blastocyst stage were transferred to foster mothers 7 days following the onset of natural estrus. Two embryos from each group (IVP or NT) were transferred per ewe. Pregnancy status was determined by ultrasonography at 40 and 60 days following transfer (Aloka, 7.5-Mhz high-resolution linear probe), and resulting pregnancies were allowed to develop to term.

Artificial Insemination and Natural Mating

As a control for recipient ewes carrying in vitro-produced or cloned embryos, the remainder of the experimental flock was either artificially inseminated (AI) or naturally mated (NM). AI was performed using frozen-thawed semen collected from the same three rams used for in vitro fertilization and for the naturally mated group.

Induction of Parturition

In order to facilitate the administration of hormones, 15 days before the induction of delivery all groups were divided. The group origin of the ewe was controlled following the delivery.

One third of the animals in each experimental and control group received no hormonal stimulation. At 146 days of gestation the remaining ewes were subjected to one of two different treatments in order to induce parturition. For the first treatment, the one-third part of each group (with the exception of the NT group) was stimulated with a single 15-mg i.m. dose of D (Dexadreson, Intervet, Boxmeer, Holland). (Previous studies [unpublished results] suggested that D was not efficient in preparing foster mothers for carrying clones for delivery. For this reason and also due to the small numbers available, the NT group was not subjected to D stimulation.)

The second treatment was developed on the basis of procedures already

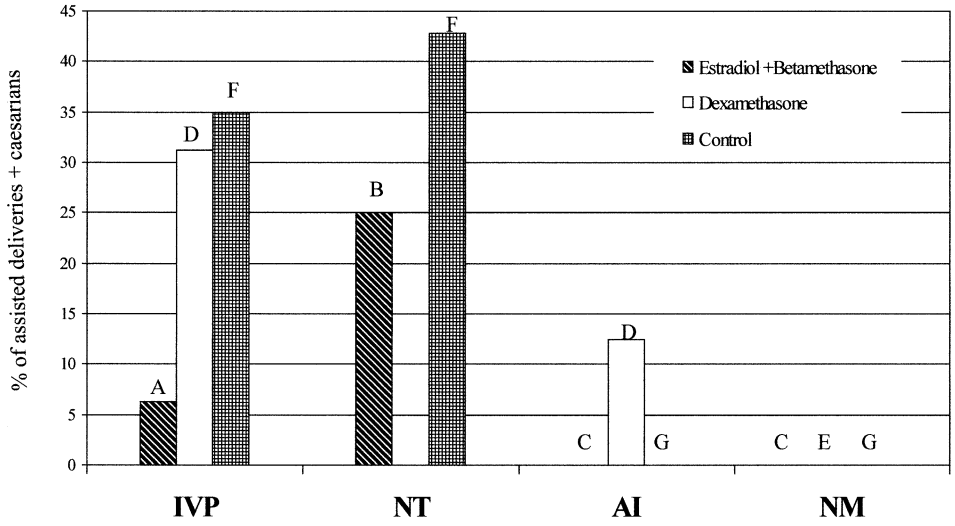

FIG. 1. Effect of reproductive procedure (IVP, NT, AI, and NM) on the occurrence of assisted deliveries and cesarians in pregnant ewes stimulated with estradiol plus betamethasone (E + B), dexamethasone (D), or nontreated control (C). Values within the same type of stimulation (E + B, D, C) are significantly different: A vs. B, B vs. C, A vs. C, F vs. G by $P < 0.001$ and D vs. E by $P < 0.01$.

in use in human neonatology units (M.G. Sanna, personal communication) and comprised a single i.m. injection of E benzoate 2 mg (Estradiolo, AMSA, Milano, Italy) followed by 4 i.m. injections of B (Bentelan, Glaxo Wellcome, Alges, Portugal) at 12-h intervals (1 mg per 10 kg body weight; E + B). This treatment also included the administration of 1.5 mg of D (Dexadreson) i.m. to the newborn lamb within minutes of delivery.

Statistical Analysis

Statistical computations were performed using chi-square analysis of all data (SAS/STAT User's Guide, SAS Institute, Inc., Cary, NC).

The observations regarding birth weight were processed by a one-way analysis of variance (ANOVA) using the statistical package SPSS Base 9.0 (1999) Applications Guide (SPSS, Inc., Chicago, IL).

RESULTS

Influence of Inducing Parturition on Delivery

All nonstimulated ewes from both the AI (n = 22) and NM (n = 55) groups delivered spontaneously without assistance. In stimulated ewes of both these groups (AI, n = 48; NM, n = 99), labor was successfully induced regardless of stimulus, although 12.5% (3/24) of AI ewes treated with D ($P < 0.05$) required assistance due to prolonged parturition (Fig. 1). The decision of intervention was made following 1 h of propulsive stage without progress in delivery.

In the nonstimulated (control) group of ewes carrying NT embryos there was an increased incidence of assisted deliveries (42.8%, 6/14; see Fig. 1), mainly as a consequence of low contractility and a general low degree of preparedness for delivery. The frequency of assistance required during delivery of the NT group decreased following the administration of E + B (25.0%, 2/8).

Similarly, a high incidence of assisted deliveries (20%, 4/20) and cesareans (15%, 3/20) was required in the group of nonstimulated ewes carrying in vitro-produced embryos. Both types of intervention resulted from a low degree of contractility and a lack of any labor signs 5–7 days after the mean term pregnancy (150 days) for Sarda sheep. The duration of parturition and the ease of lambing was not improved by D with the proportion of prolonged deliveries similar to the control group (31.3%, 5/16 vs. control: 35.0%, 7/20). The requirement for parturient assistance in the IVP group diminished following E + B (6.3%, 1/16), where the preparedness for parturition (behavior, contractility) and ease of delivery was similar to naturally generated offspring. The single intervention during delivery of the IVP group was due to the poor condition of that particular foster mother (advanced age, pneumonia), rather than a result of difficulties specific to IVP and NT pregnancies.

As expected, there was considerable variation in the delivery time in all groups of nonstimulated ewes (144–159 days of gestation), although all pregnancies were within the normal range for the Sarda breed. Mean pregnancy length was relatively high for the nonstimulated NT group (154.9 days), decreased for the nonstimulated IVP group (152.2 days), and was typical for the Sarda breed mean values for both the AI (149.6 days) and the NM (149.8 days) groups. In all groups both treatments significantly influenced the time of delivery (see Fig. 2); however, E + B stimulation more precisely controlled the timing of parturition. The delivery time range was 36–53 h post-combined (E + B) injection, whereas a much wider range (36–168 h postinjection) was observed following the D injection.

Twin pregnancies had no effect on gestation length or on parturition performance compared to single gestations, and as detailed later, the lack of behavioral and physiological signs of approaching parturition was not a function of number of lambs carried. Overall, there were no significant differences in the proportion of twin pregnancies between any of the IVP, NT, AI, and NM groups (23.5% vs. 28.2% vs. 30.5% vs. 24.0%, respectively).

Intrapartum Characteristics of Ewes

In the majority (68%, 23/34) of nonstimulated pregnant ewes that received IVP and NT embryos, some of the physiological and behavioral symptoms of preparedness for delivery were disrupted. The most common signs were lack of edema of the vulva and insufficient cervical ripening. In addition, there was either a total lack of contractility or poor contractility during expulsion of the lamb. In more than half (59%, 20/34) of all nonstimulated foster mothers, preparation of the mammary gland, the first characteristic sign of a ewe nearing delivery, did not occur.

Moreover, intra- and postpartum maternal behavior was severely disturbed. Common symptoms of a ewe preparing for delivery, such as increased motional activity, bleating, and searching for lambs were not usually observed in both IVP and NT nonstimulated groups of parturient ewes. In extreme cases, foster mothers continued to eat during delivery. Onset of licking was delayed or failed to occur, and maternal bleating was severely reduced. The commence-

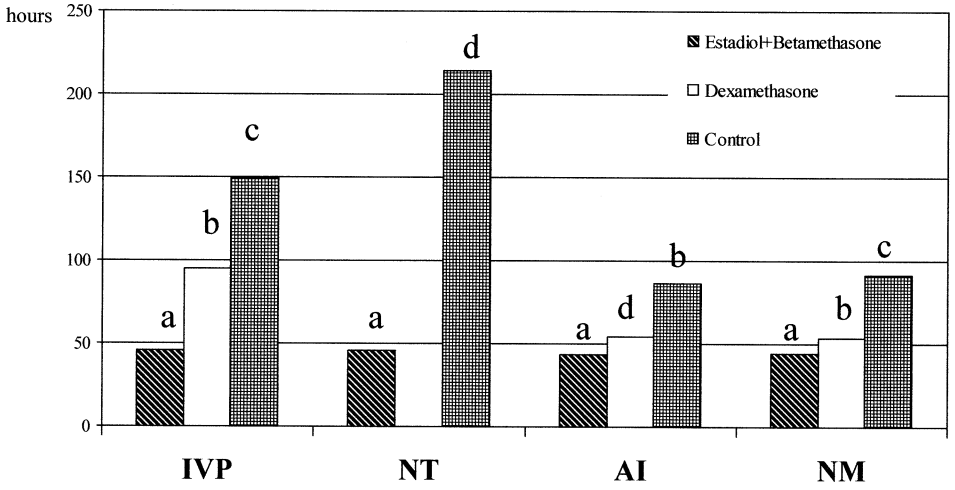

FIG. 2. Effect of hormonal treatment on the mean time of delivery of ewes carrying pregnancies obtained by IVP, NT, AI, and NM (measured from 0900 h, Day 146 of gestation). Values within the same group (IVP, NT, AI, NM) are significantly different: a vs. b, a vs. c, b vs. c, b vs. d by $P < 0.01$ and a vs. d by $P < 0.05$.

ment of suckling was also delayed, although this could be attributed to the condition of the lamb. Such maternal behavior could be a consequence of the physiological failure to prepare for parturition. Overall, the proportion of mothers failing to display an immediate onset of maternal care (in <5 min) was significantly higher ($P < 0.001$) in nonstimulated IVP (16/20) and NT ewes (14/14) compared to both AI (none of 22) and NM (1/55) ewes, whereas there were no significant differences between nonstimulated IVP and NT recipients.

All of the physiological and behavioral peripartum irregularities (disturbances) described diminished significantly ($P < 0.001$) when parturition was induced by E + B (IVP, 1 of 16; NT, 2 of 8), but not with D (IVP, 12 of 16).

Offspring Outcome

The mortality of offspring within the same treatments differed significantly between artificially generated embryos (IVP and NT) and controls (AI and NM; see Fig. 3), whereas no significant difference in offspring mortality was observed between IVP and NT groups stimulated with E + B or left untreated. Similarly, no differences in offspring viability within the same treatment were noted between both controls (AI and NM).

A highly significant effect on the reduction of mortality ($P < 0.001$) was revealed between those stimulated with E + B and those stimulated with D within the group of in vitro-derived offspring. It is worthy of note that in the same group of animals (IVP) there were no differences in offspring mortality between nonstimulated ewes and those stimulated with D. Similarly, important improvements (although not significant because of the small number of animals involved) in the viability of offspring were observed in the NT group stimulated with E + B. Such positive effects of E + B treatment on lamb viability was not statistically confirmed for AI and NM groups, notwithstanding the substantial number of animals represented in both these control groups.

There were no significant differences in the sex ratio (number of females/number of males) of newborns in the IVP, AI, and NM groups, respectively: 31/37, 47/54, and 97/106. In the NT group two sets of five male clones, one set of four female clones, one set of three male clones, one set of three female clones, and one set of two female clones were born. The mean birth weight of lambs was not influenced by the type of treatment (E + B, D, C) or by group (IVP, NT, AI, NM; data not shown); however, the ANOVA reveals a highly significant effect ($P < 0.001$) of two variables: sex and number (single/twins) of lambs on their birth weight within IVP and NT groups and significant effect of number (single/twins) of lambs within the AI group ($P < 0.001$).

DISCUSSION

The glucocorticoids administered to pregnant ewes provoke the modification of placental steroidogenesis that physiologically proceeds the parturition. Thus they reproduce the action of fetal cortisol, which reaches the placenta with the fetal blood circulation. In general, corticoids can induce parturition near term, and their efficacy depends on their attitude to pass the placenta. In swine and equine species, very low placental permeability does not allow induction of the delivery while it is possible in ruminants. In ruminants, and particularly in sheep, the prepartum surge in fetal cortisol stimulates placental steroidogenic enzymes that initiate the fall in maternal plasma progesterone and lead to the final hormonal cascade. Exogenous glucocorticoids mimic the fetal prepartum surge, causing a gradual fall in progesterone and concomitant changes in placental estrogen and prostaglandin (PG) before labor begins. The secretion of estrogens, however, often remains more weak with respect to that in natural parturition [32]. It is only recently that the underlying endocrine mechanisms have been sufficiently understood to explain uterine activity. In the sheep, it has recently been proposed [33] that the prepartum increase in fetal adrenal cortisol output within trophoblast tissue leading to PGE_2 production occurs independently of an increase in placental estradiol (E_2) output, whereas the E_2-dependent pathway within the maternal endometrium leads to $PGF_{2\alpha}$ output, and as a consequence, myometrial contractility is stimulated and labor and delivery ensue. Thus the failure of adequate parturition performance and especially insufficient contractility can be associated with low E levels during corticosteroid induction of parturition.

The induction of parturition with the aid of glucocorticoids has been already performed in sheep using either B and D i.m. injections [34]. Both molecules have very similar structures, differing only in stereoisomery of their C-16 methyl group. As fluorinated steroids, both epimers are more effective in accelerating lung maturation than less potent cortisol, cortisone, and prednisone. Both B and D can successfully induce delivery; however, the expression of the enzymes of E synthesis (placental cytochrome 17α-hydrox-

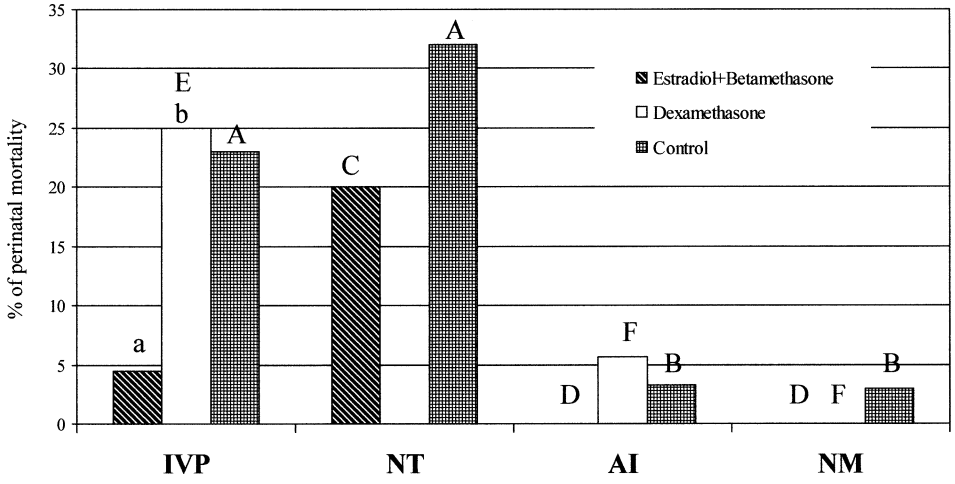

FIG. 3. Effect of hormonal treatment on the mortality of offspring obtained by in vitro production (IVP) of embryos, nuclear transfer (NT), artificial insemination (AI), and natural mating (NM). Values A and B within the same group (IVP) are significantly different by $P < 0.001$. Values A, B, C, D, E, F within the same type of treatment (E + B, D, C) are significantly different: A vs. B, C vs. D, E vs. F by $P < 0.01$.

ylase) associated with spontaneous term labor, and thus a more physiological way of action, is observed following B (and not D) administration [35].

Here we assessed the effects of two separate hormonal regimes on delivery performance and offspring viability following transfer of IVP and cloned sheep embryos. One of these regimes, a single injection of D, has been in common use for naturally mated females for around 30 years [28], although recent studies have identified several defects associated with D treatment. In sheep, D will eventually induce labor, although the timing is imprecise [36]; however, its use may also alter the composition and volume of fetal fluids [37] and provoke temporal changes in the fetal cardiovascular system [38]. These observations have also been extended to other species such as rats [39, 40], guinea pigs [41], and rhesus monkeys [42]. Our second hormonal regime consisted of one fixed dose of E followed by four injections of B (E + B). Recent studies have confirmed the potent action of B in inducing the essential mechanisms of parturition in sheep [35, 43], and the use of E benzoate to induce parturition either alone [27] or in combination with glucocorticoids [32] has also been described.

In our study the combined treatment of E and glucocorticoids (E + B) improved the performance of several pre- and postpartum events in both experimental groups (IVP, NT). First, a significant decrease in the requirement for human intervention (assisted deliveries and cesareans) was observed during the parturition of foster mothers (see Fig. 1). In contrast, when using the D treatment the number of assisted deliveries was similar (IVP) or even significantly higher (AI) than for nonstimulated ewes from corresponding groups. A greater number of interventions during deliveries stimulated by D compared to B stimulation had previously been observed in parturient ewes in addition to a slightly longer interval from induction to lambing [34]. Here the time of delivery was also extended when using D (up to 168 h postinjection) compared to the more restricted period associated with B + E treatment (all within 53 h).

In both nonstimulated experimental groups (IVP and NT), the majority of physiological and behavioral signs of preparedness for delivery were disrupted. Some of these signs, such as impaired labor (weak propulsive stage) and little preparation of the mammary gland, have already been described following transfer of both IVP bovine [25] and NT [3, 44] ovine and bovine embryos. Our previous studies revealed a failure of maternal contribution to the labor of recipients carrying IVP and NT pregnancies [45–47], and here we observe further physiological deficiencies during the initial phase of parturition, including a lack of or insufficient edema of the vulva and a low degree of ripening of the cervix. Another important indicator of approaching parturition, characteristic maternal behavior, was also severely affected (see *Results*). D treatment did not restore the majority of indicators of imminent delivery, and it actually prolonged parturition of artificially inseminated ewes (see Fig. 1). Recent studies performed on a substantial number of ewes carrying embryos obtained from routine multiple ovulation/embryo transfer also revealed a high frequency of prolonged parturitions in ewes stimulated with D (M. Dattena, personal communication). In cattle, D-induced parturition has been associated with an increased incidence of retained placenta [32]; however, this can be explained by the effects on E levels. These older studies show a lower circulating plasma E level at parturition following treatment with D compared with levels in animals delivering spontaneously [32]. More recent experiments confirm that D decreases basal levels of maternal E and abolishes the prepartum estrogen and prolactin surges [42]. It is well documented that E is an important endocrine factor that stimulates maternal responsiveness both in nonpregnant and in parturient ewes [48, 49]. As the induction of maternal responsiveness in sheep is steroid-dependent, it is possible that the suppression of estrogen biosynthesis in D-treated ewes and, more importantly, inadequate signaling between NT or IVP fetus and foster mother may be responsible for impaired pre- and immediate postpartum behavior.

The major achievement reported here is in overcoming the physiological and behavioral intrapartum failures of foster mothers using a combination of E and glucocorticoid (E + B) injections. Here we confirm previous findings that demonstrated that B is more efficient than D at inducing labor in sheep [43]. Furthermore, E-dependent myometrial activation before and during labor appears to play a critical role in aligning fetal signals of preparedness for birth with maternal factors promoting labor [50]. One of the most important consequences of the E + B treatment is the positive effects on offspring viability in both experimental groups (IVP and NT). In contrast, D stimulation alone resulted in a slight increase in IVP and AI offspring mortality compared to their respective nonstimulated controls (see Fig. 3).

Regulation of many of the final preparations for birth is under the control of the fetal adrenal glands, and accordingly proper glucocorticoid administration is important in

accelerating appropriate fetal and maternal prepartum events. A successful transition from intrauterine to extrauterine existence is dependent on events that precede birth: functional maturation of the lungs, laying down of carbohydrate and fat reserves, etc. Preparedness for parturition also requires fetal and placental production of hormones required to induce the onset of lactation. In sheep, lactation can precede labor by a variable number of days, thereby enabling the newborn to obtain food at birth. Our protocol of parturition induction using a combination of glucocorticoids and E injections ensured the correct preparedness for delivery in foster mothers, thus ensuring more adequate fetal pulmonary and glucogenic maturation and improved development of the maternal mammary gland, which lead to the proper adaptation of offspring following birth.

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