The Texas Journal of Agriculture and Natural Resource 20:52-62 (2007)

EFFECTS OF PROTEIN AND ENERGY FEEDING ON OVINE OOCYTE PRODUCTION AND DEVELOPMENTAL CAPACITY

W. A. Kiker
M. W. Salisbury
B.J. May
G. R. Engdahl
Department of Agriculture, Angelo State University, San Angelo, Texas

ABSTRACT

A study was conducted to determine the effects of protein and energy on oocyte production in ewes. Eighty-one multiparous whiteface ewes were randomly divided into three feeding treatments, and penned in groups of three, which served as the experimental unit. Ewes were placed on one of three feeding treatments, wheat hay (maintenance), mixed grain with added protein, or mixed grain with added energy. Ewes were fed for 35 d then ovaries were removed, trimmed, weighed, lacerated, and rinsed with TL Hepes. Recovered oocytes were graded and matured in the incubator for 24 h in 5% CO_2 at 38.5°C. Following incubation, oocytes were removed, rinsed and activated to begin development. Oocytes were incubated in a blood gas mixture for 7 d and evaluated at d 4 and d 7 for cleavage rates and morula formation. Results yielded no difference ($P > 0.05$) among feeding treatments with respect to ovarian weights, oocyte numbers, quality scores, or developmental rates.

Key Words: Sheep, Oocytes, Ovaries, Protein, Energy

INTRODUCTION

Reproductive capabilities of livestock have long been associated with their nutritional state. The methods in which nutrition, genetics, health, and reproduction correlate together are not well understood (Ayalon, 1978). Animals are placed into a negative nutritinal balance when they do not receive enough energy or protein in their diet to meet the demands of energy expenditure for locomotion, milk production, reproduction, and maintenance (Dunn and Moss, 1992). Once an animal cannot meet its maintenance needs, it begins to falter in other areas such as reproduction in order to maintain its body functions to survive.

Angora nannies that were flushed for five weeks with additional energy in their diet just prior to mating showed improved conception rates over the nannies that were not flushed. Even nannies that were not flushed but simply put on a higher level of feed were able to improve their body condition enough within five weeks of breeding to conceive (Taylor et al., 1988a). Taylor et al. (1988b) conducted another study that involved

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subjecting angora nannies to nutritional stress for a period of three days, then placing them back on free feeding. They believed that this fast-then-feed method raised glucose levels to stimulate ovulation. What role the physiological aspects might have played in the increased ovulation or conceptions of the studies is unclear; however it is apparent that nutrition is a key component to reproduction. Hunt et al. (1988) produced results that concur with Taylor et al. (1988) and showed non-significant trends in ovulation increases when ewes were flushed.

Research has shown increased amounts of protein in the diet of dairy cows can have a detrimental effect on their reproductive success (Jordan and Swanson, 1979). Elrod and Butler (1993) found a decrease in the rate of pregnancy from 82% in the normal feeding group to 61% in the group of heifers fed high levels of protein. Protein excesses have continually shown a deleterious effect towards reproductive performance. Energy feeding has also been shown to affect reproductive traits in cattle. Butler and Smith (1989) state that energy can be a limiting factor on reproductive performance in postpartum cows. Wiltbank et al. (1965) stated energy limitations could adversely affect reproductive success of beef cattle more than protein. However, an increase in dietary energy seems to enhance reproduction while protein inhibits reproductive ability. Studies by Gwazdauskas et al. (1999) and Kendrick et al. (1999) both showed that cows fed high-energy diets produced more overall good oocytes than cows fed low energy diets. Contrary to these findings, Nolan et al. (1998) and Callaghan et al. (2000) found nutrition had no effect on the morphological grading of oocytes.

It is essential that the nutritional affects on the ovary and the substrates of the diets be further evaluated to try and close the gap in information. Not only is it important that we study the effects on the embryos of nutritional stress and dietary changes, but the levels before embryo development must also be further evaluated. Therefore, this study was designed to evaluate the effects of protein and energy on oocyte production, quality and developmental capacity in white-face ewes.

MATERIALS AND METHODS

Animals and Dietary Treatments

Eighty-one multiparous whiteface ewes were randomly divided into three groups of 27. Ewes were fed in groups of three and each group of three ewes formed an experimental unit. Treatments consisted of: a mixed grain diet containing more available energy (Treatment 1), a mixed grain diet containing more protein (Treatment 2), or a maintenance diet of wheat hay (Treatment 3) intended to meet only maintenance needs (Table 1). Ewes were fed at a rate of 2.5% of body weight to meet the NRC recommended amount of total digestible nutrients for maintenance. One ewe died and the feeding ratio was adjusted accordingly for that pen. Treatment 1 consisted of a higher amount of corn (19.6% vs. 9.7%) to add energy to the diet, than the protein diet, which had a higher level of cottonseed meal (11.8% vs. 3.1%), to add protein to the diet.

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Table 1. Ingredients and nutritional analysis, DM basis, of dietary treatments fed to ewes at 2.5% of body weight per day.[a]

	Treatment[b]		
Ingredients, %	1	2	3
Corn Grain	19.6	9.7	0.0
Soybean Hulls	30.4	30.2	0.0
Sheep Premix	2.7	2.7	0.0
Cane Molasses	4.2	4.2	0.0
Cotton Hulls	40.0	41.3	0.0
Cotton Seed Meal	3.1	11.8	0.0
Wheat Hay	0.0	0.0	100.0
Nutrients, %			
Crude Protein	12.1	15.9	19.6
Total Digestible Nutrients	71.0	72.0	59.0
Acid Detergent Fiber	42.4	43.1	29.7
Neutral Detergent Fiber	51.8	48.6	51.6

[a] Randomized complete block design with pen of three ewes serving as the experimental unit
[b] Treatments consisted of 1, energy diet; 2, protein diet; 3, wheat hay.

Before the ewes were assigned to their dietary trial, they were fed a diet of strictly wheat hay that meets the NRC requirements for maintenance to allow them to adjust to the environment (adjustment). For purposes of time management, the three groups of ewes were staggered one week apart when placed on their respective diets. Three subgroups, consisting of three ewes each from each feeding treatment, were placed on their dietary rations. Due to the amount of time involved in the harvesting of the ewes and the collection and processing of their ovaries, it was necessary to divide the overall feeding, collection, and processing into thirds. Therefore, the first subgroup was on the adjustment diet for two weeks, the second group for three weeks, and the third group for four weeks. Once assigned to their feed trial period, all ewes stayed on their designated feed for five weeks.

Ovary Retrieval

Upon the conclusion of the five-week feeding trial, each set of ewes was taken to Ranchers' Lamb of Texas Inc. (San Angelo, Texas) for harvest. Ovaries from each ewe were removed by manually pinching them off the reproductive tract. They were immediately placed in phosphate buffered saline for transportation to the laboratory for processing. The ovaries were trimmed of excess tissue and a weight was taken on a per pen basis.

Cumulus Oocyte Complexes Retrieval

Protocols used for cumulus oocyte complexes (COC) retrieval, oocyte maturation, storage and culture, were provided by Ovagenix Laboratories (East 37th Street San Angelo, Texas 76903). Ovaries were sliced with a scalpel blade and rinsed with TL

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Hepes, modified Tyrode's medium with 0.03% bovine serum albumin fraction V (BSA), commonly used for most embryo and oocyte holding and culture media. The ovaries were then chopped and placed in a 50 ml conical tube (Fisher Scientific, Houston, TX) with 10 mls of TL Hepes, shaken, then drained and rinsed into another 100 ml petri dish. The COC's were removed from the original dishes and placed in a clean dish of TL Hepes for rinsing and sorting. Cumulus oocyte complexes were counted into a third dish for a third wash and grouped according to quality.

Oocyte Grading

Quality scores were based on those of Deloose et al. (1989) and Tripp et al. (1999). Scores ranged from A (excellent) to D (poor) based on cumulus orientation and ooplasm regularity. Category A consisted of oocytes that are surrounded by at least 3 layers of compact cumulus cells and contained a normal cytoplasm. Cumulus oocyte complexes scoring a B were surrounded by 2 layers of compact cumulus cells and held a normal cytoplasm. Category C contained oocytes surrounded by 1 layer of compact cumulus cells and contained a normal cytoplasm. Category D included COC's with less than 1 layer of cumulus cells or an unstable layer of cells with an abnormal cytoplasm. Normal cytoplasms appeared smooth and complete in structure without defects. Abnormal cytoplasms had color blotches and voided areas. The COC's scoring A and B were grouped together for maturation, culture, and overall assessment. The COC's scoring C were kept alone, and the D scoring groups were disposed of, as it was highly unlikely that the D groups would be capable of maturation (Deloose et al. 1989).

Oocyte Maturation

The COC's were placed in a maturation medium containing 88% M199 with Earle's salts (Gibco), 0.5% luteinizing hormone (LH), 0.5% recombinant bovine follicle stimulating hormone (bFSH), 10% fetal bovine serum (FBS), and 1% penicillin/streptomycin and placed in incubation with 5% CO_2 at 38.5˚C for 24 h. The M199 is a complex cell culture medium used as the base medium for oocyte maturation and embryo culture, and Earle's salts is bicarbonate buffered and must be used in an atmosphere of 5% CO_2 to maintain proper pH (Ovagenix Laboratories). Luteinizing hormone, bFSH, and FBS were used in oocyte maturation. This procedure was done to mature the COC's relatively close to the same developmental stages and equilibrate them into the new environment outside of the follicle (Ovagenix Laboratories).

Oocyte Rinsing and Storage

After 24 h of incubation, the matured oocytes were removed and placed in 1 ml of TL Hepes in a 15 ml conical tube. The oocytes were vortexed at high speed for 2 min 15 s to remove cumulus cells and reveal the actual oocyte. The oocytes were rinsed from the conical tube into a 60 mm petri dish with TL Hepes. The oocytes were rinsed using a multiple step activation protocol. First, they were rinsed in a combination of 5% ionomyocin in TL Hepes for four min to disinfect them from any outside contaminants. The lights were turned down to a minimum and the ionomyocin was kept covered at all possible times since it is light sensitive. Second, the oocytes were rinsed in a wash of TL Hepes for four min to remove ionomyocin debris. Third, they were rinsed in a wash of M199 with 10% FBS and 10% 200 mM dimethlyaminopurine (DMAP; Genetic Savings and Clone, Bryan, TX) to remove all other liquid associates. Finally, they were stored in

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a fresh mixture of M199 with 10% FBS and 10% 200 mM DMAP for 5 h at 38.5°C with a 5% CO_2 atmosphere. DMAP was added to the medium to maintain oocytes in a state of meiotic arrest once they have been removed from the follicle. The addition of DMAP allowed the oocytes to be incubated at a fixed point in development without advancement beyond the stage of initiation. This allowed time for the oocytes to re-equilibrate with the holding environment before they were activated for culture.

Oocyte Culture

At the completion of 5 h, the oocytes were removed from the incubator, then rinsed and stored in a solution called Barc's (Genetic Savings and Clone, Bryan, TX) designed to cause the oocytes to undergo cleavage and divide as if they had been fertilized and become embryos. The oocytes were cultured for 7 d at 38.5°C in a medical grade, blood gas mixture containing 90% nitrogen, 5% carbon dioxide, and 5% oxygen (Airgas, San Angelo, TX) to enhance tissue development and growth. After 4 d of culture, the oocytes were removed and evaluated for cleavage rates. After the oocytes were evaluated and the findings recorded, they were placed back into the gas mixture and cultured for the remaining 3 d. Once the oocyte completed the full 7 d culture period, they were removed and assessed for morula formations, and blastocyst development. Oocytes evolving to blastocyst were considered fully developed because that is as far as an oocyte can transform without the assistance of DNA from sperm.

Data Collection and Procedures

Collection and handling procedures of ovaries and oocytes were similar to the methods of Rho et al. (2001) and Khurana and Niemann (2000). Body weights were taken prior to the initiation of each groups' feeding trial and after the completion of each trial before harvest. Means were calculated and recorded according to experimental units and feed groups. Totals were recorded for ovarian mass, oocyte production, oocyte grades, and oocyte development for each of the treatment groups.

Statistical Analysis

Pen of three ewes was considered the experimental unit. Day of harvest was included in the model to account for any differences in day associated with the ewes completing the experiment on different weeks. Body weight, ovarian weight, oocyte numbers (total and per classification), percent cleavage rates and development rates (morula and blastocyst) were analyzed using the General Linear Models of SAS (SAS Inst., Inc., Cary, NC.). Duncan's Least Significant Difference procedure was performed to separate mean differences. Treatment differences were considered different at $P < 0$ .05.

RESULTS AND DISCUSSION

Animal Performance

There were no difference ($P > 0.05$) in weight gain or loss among the different treatment groups in relation to oocyte production. Neither ovarian weights, oocyte production, oocyte grades, nor oocyte development were affected ($P > 0.05$) by weight gain or loss of the ewes.

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Dietary Treatments

Feed analysis were performed by Dairy One DHI Forage Testing Laboratory and the results were: treatment 1 contained 19.6% corn grain on a DM basis, where treatment 2 contained only 9.7% corn grain on a DM basis. Treatment 3's energy availability was derived from the nutritional value of the wheat hay with no supplementation (Table 1). After analysis, the quality of the hay was higher than anticipated. Thus, the maintenance diet was not present, but two diets had protein levels above those reported for maintenance. The differences were then forage versus mixed grain.

Ovarian Weights, Oocyte Totals, Grade Percentages, and Development

No differences ($P > 0.05$) in ovarian weights, oocyte totals, oocyte grades (Table 2), or blastocyst development were seen among treatment groups (Table 3). No differences were found that would indicate nutritional levels: 1) affected the way the ovary grows due to follicular or tissue development, 2) caused the oocyte to produce more primary, secondary, or tertiary follicles, 3) resulted in a higher or lower abundance of retrievable oocytes, 4) produced varying amounts of A, B, C, or D quality oocytes, 5) influenced the developmental capacity of the oocytes to reach the blastocyst stage.

Table 2: Ovarian weights, oocyte totals, and oocyte grade percentages from ewes offered three dietary treatments.[a]

	Treatments[b]			
	1	2	3	Standard Error
Ovarian weight, g	10.76	9.72	10.42	0.476
Oocyte totals, g	39.78	47.11	55.00	6.968
Percent A/B	41.56	46.89	38.43	3.68
Percent C	19.27	20.15	21.51	1.73
Percent D	39.56	32.95	36.86	3.15

[a] Randomized complete block design with pen of three ewes serving as the experimental unit.
[b] Treatments consisted of 1, energy diet; 2, protein diet; 3, wheat hay.

Table 3. Percentages of oocyte cleavages, morulas, and blastocyst formations from ewes offered three dietary treatments.[a]

	Treatments[b]			
Item, %	1	2	3	Standard Error
A/B Cleavage	92.75	91.42	81.88	4.84
A/B Blastocysts	60.60	57.29	51.82	6.60
A/B Morulas	8.35	11.62	9.71	2.54
C Cleavage	72.09	80.69	73.21	7.58
C Blastocysts	41.54	37.00	21.62	8.05
C Morulas	10.41	19.85	10.27	6.26

[a] Randomized complete block design with pen of three ewes serving as the experimental unit
[b] Treatments consisted of 1, energy diet; 2, protein diet; 3, wheat hay.

One area of interest that was not approached in this study was the feeding of different quantities and the effect that it has on ovary production. Papadopoulos et al.

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(2000) conducted a study where they fed grass meal to sheep at either 2.0 times maintenance energy requirements (MER) or 0.5 times MER. They found that ewes on the 0.5 MER diet produced less overall follicles than the ewes on the 2.0 MER diet. Despite these findings, no difference in the rate of oocyte retrieval due to feeding amounts was observed. Animals on the 2.0 MER did have a lower cleavage rate than those on the 0.5 MER diet; however, no differences in blastocyst rate, blastocyst-hatching rate, or blastocysts cell number were seen when expressed as percentage of cleaved oocytes. The development of more follicles in their study could affect the weights of the ovaries whereas in our study, the weights were unaffected by the three feeding groups. The altering of the amount of the grain diets and forage diet could have shown a difference in the weights of ovaries retrieved due to follicle production. Similar to these results, our study did not show a difference in the total number of oocytes derived from the three treatment groups. This evidence suggests that neither feeding amounts, nor the type of feed affects the total oocyte production of the ovary. Papadopolous et al. (2000) concurs with the current study that neither different feeding amounts, nor nutritional amounts or types affect the development of oocytes after cleavage occurs. Callaghan et al. (2000) also supports these findings. When crossbred ewes were assigned to one of three diets and fed levels of 0.5, 1.0, or 2.0 times maintenance energy requirements, no difference in oocyte morphology was found associated with the feeding trials. Another study corresponding to these was conducted by Nolan et al. (1998). Heifers were fed an *ad libitum* or restricted diet of grass silage/concentrate at a 10:1 ratio. These feeding treatments had no apparent effect on oocyte grades or oocyte development in an *in vitro* setting. Results appear to be conclusive that feeding amounts do not effect ($P > 0.05$) the quality and development of oocytes but do affect the ovulation rates as expressed by Henniawati and Fletcher (1986) in an experiment with Indonesian sheep and goats. Sheep and goats were placed on either a maintenance level or a supramaintenence level of nutrition containing elephant grass and a supplemental commercial concentrate. The supramaintenece diet showed a strong improvement of ovulation over the maintenance diet. Research has shown that ovulation rates and follicle formation can be affected by feeding amounts to allow for an area of improvement in reproduction.

Other research supports the evidence that differences in ewes offered half maintenance energy requirement diets vs. ewes offered double the maintenance energy requirements is insignificant in regards to oocyte morphology (Boland et al. 2001). However, other evidence suggests a relation between the *in vitro* development of oocytes from harvested heifers that were restricted from energy intake prior to slaughter (McEvoy et al., 1997). McEvoy et al. (1997) also found that the blastocyst yields from heifers on low energy intake rather than high-energy intake were enhanced by the nutritional diets. Nolan et al. (1998) also supports these results in their study with enhancement of oocytes collected trans-vaginally to blastocyst development *in vitro* from heifers restricted of dietary intake. These research studies further emphasize the role that nutrition plays on reproduction is very important. Enough evidence exists to suggest a relationship between nutrition and reproduction at the level of the oocyte.

Feeding different types of diets, forages or concentrates, may be capable of producing different outcomes in regards to oocyte production. An experiment was conducted on heifers that were fed either a diet of barley concentrate or a diet of a citrus/beet pulp mixture. Both feeds were designed to contain 14% crude protein. Heifers on the citrus/beet pulp diet produced more freezable and transferable embryos

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than heifers on the barley diet. However, the numbers of retrieved embryos with lower grades, and unfertilized ova were not affected by concentrate type (Yaakub et al., 1998). This study did not take into account different amounts of nutritional protein in the diet, or energy levels, nor did it evaluate the results of the diets used on the production and development of oocytes. The information provided does give an implication that a different effect was found on the production of embryos according to the feed types. This insinuates a possible impact on the ovaries or the oocytes before the time of conception. It is possible a greater number of quality oocytes were produced that led to a larger amount of quality grade embryos from the feeding of citrus/beet pulp as compared to the feeding of barley concentrate. This would give hope to the assumption that the production and development of oocytes can be affected by the influence of the type of feed and the nutritional components of the feed.

An abundance of data exists revealing connections with reproduction and nutrition at the level of the ovary and the oocyte. While some of the evidence is contradictory, it all shows a connection in one way or another. Whether it is the effect of nutrient deficiencies, feeding abundances, or different types of feeds involved, many of these studies show an effect on the production of the ovary and the oocyte. Some of the studies show increases in follicular production and enhanced oocyte developmental capacities, and others provide information contradicting these findings showing decreases in follicular production and oocyte developmental capacities. Several of the studies indicated decreases in the nutritional feeding of animals causes a positive outcome with ovarian production, and some insist that enhanced feeding is required to reach the same effects. A few of the studies even offer the explanation that energy is beneficial to reproductive success and protein is deleterious to reproductive success. It is apparent that the dynamics of nutritional effects on reproduction are still not fully understood. So many of the studies offer contradictory findings that a definite need for further investigation in the search for answers regarding nutritional effects on reproductive success exists.

CONCLUSIONS

The primary goal of this study was to determine if added protein or energy to a maintenance diet would increase oocyte production rates, oocyte quality or developmental capacity in mature ewes, therefore enhancing the reproductive rate and success of sheep. This study did not take into account physiological characteristics or digestibility factors of different types of feeds. It also did not address the issues of nutritional stress from limit feeding or the effects of over feeding. These are areas that should be further explored as a large amount of research exists that indicating these areas have an effect on reproduction in one way or another.

Based on the literature reviewed, we hypothesized an increase in protein would have detrimental affects on oocyte production through decreased numbers, quality, and survivability, but energy increases should have shown beneficial results in the respective fields of oocyte production. This hypothesis was not supported in this study. We did not see differences in any of the respective areas in association to the diets used.

Results of this study indicate that increased amounts of protein in a ewe's diet will not increase oocyte production or developmental capacity thus not having an effect

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on conception rate. Therefore, it may prove more economically efficient for producers to feed energy instead of protein to ewes during the breeding season since energy is more cost efficient than protein. Also, researchers in the field of oocytes may not need to account for differences in nutritional values of feeds when evaluating the developmental capacities of oocytes from ewes when maintenance requirements are met by the diet.

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REFERENCES

Ayalon, N. 1978. A review of embryonic mortality in cattle. J. Reprod. Fertil. 54:483-493.

Boland, M. P., P. Lonergan, and D. O'Callahan. 2001. Effect of nutrition on endocrine parameters, ovarian physiology, and oocyte and embryo development. Theriogenology. 55:1323-1340.

Butler, W. R., and R. D. Smith. 1989. Interrelationships between energy balance and postpartum reproductive function in dairy cattle. J. Dairy Sci. 72:767.

Callaghan, D. O., H. Yaakub, P. Hyttel, L. J. Spicer, and M. P. Boland. 2000. Effect of nutrition and superovulation on oocyte morphology, follicular fluid composition and systemic hormone concentrations in ewes. J. Reprod. Fertil. 118:303-313.

Dunn, T. G., and G. E. Moss. 1992. Effects of nutrient deficiencies and excesses on reproductive efficiency of livestock. J. Anim. Sci. 70:1580-1593.

DeLoose, F., C. Van Vliet, P. Van Maurick, and T. A. M. Kruip. 1989. Morphology of immature bovine oocytes. Gamete Res. 24:197-204.

Elrod, C. C., and W. R. Butler. 1993. Reduction of fertility and alteration of uterine pH in heifers fed excess ruminally degradable protein. J. Anim. Sci. 71:694-701.

Gwazdauskas, F. C., K. W. Kendrick, A. W. Pryor, and T. L. Bailey. 1999. Impact of follicular aspiration on folliculogenesis as influenced by dietary and stage of lactation. J. Dairy Sci. 83:1625-1634.

Henniawati, and I. C. Fletcher. 1986. Reproduction in indonesian sheep and goats at two levels of nutrition. Anim. Reprod. Sci. 12:77-84.

Hunt, L. J., J. E. Huston, B. S. Engdahl, and K.W. Bales. 1988. Effects of flushing ewes in three different body conditions on ovulation and lambing rates. Sheep and goat, wool and mohair. Texas Agricultural Experiment Station. 6-7.

Jordan, E. R., and L. V. Swanson. 1979. Serum progesterone and luteinizing hormone in dairy cattle fed varying levels of crude protein. J. Anim. Sci. 48:1154-1158.

Kendrick, K. W., T. L. Bailey, A. S. Garst, A.W. Pryor, A. Ahmadzadeh, R. M. Akers, W. E. Eyestone, R. E. Pearson, and F. C. Gwazdauskas. 1999. Effects of energy balance on hormones, ovarian activity, and recovered oocytes in lactating holstein cows using transvaginal follicular aspiration. J. Dairy Sci. 82:1731-1740.

Khurana, N. K., and H. Niemann. 2000. Effects of oocyte quality, oxygen tension, embryo density, cumulus cells and energy substrates on cleavage and morula/blastocyst formation of bovine embryos. Theriogenology. 54:741-756.

McEvoy, T. G., K. D. Sinclair, M. E. Staines, J.J. Robinson, D. G. Armstrong, and R. Webb. 1997. In vitro blastocyst production in relation to energy and protein intake prior to oocyte collection. J. Repro. Fertil. 19:132 abst.

Nolan, R., D. O. Callaghan, R. T. Duby, P. Lonergan, and M. P. Boland. 1998. The influence of short-term nutrient changes on follicle growth and embryo production following superovulation in beef heifers. Theriogenology. 50:1263-1274.

Nolan, R., P. Duffy, M. Wade, D. O'Callaghan, and M. P. Boland. 1998. Effect of quantity and type of diet and frequency of trans-vaginal ovum aspiration on *in-vitro* embryo development in heifers. Theriogenology. 49:402 abst.

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Papadopoulos, S., P. Lonergan, V. Gath, K.M. Quinn, A.C.O. Evans, D.O'Callaghan, and M.P. Boland. 2000. Effect of diet quantity and urea supplementation on oocyte and embryo quality in sheep. Theriogenology. 1059-1069.

Rho, G. J., A. C. Hahnel, and K. J. Betteridge. 2001. Comparisons of oocyte maturation times and of three methods of sperm preparation for their effects on the production of goat embryos *in vitro*. Theriogenology. 56:503-516.

Taylor, C. A. Jr., N. E. Garza. Jr., J. E. Huston, R. A. Moen, and T. D. Willingham. 1998a. Reproductive response of angora nannies in confinement to changes in diet quantity. Sheep and goat, wool and mohair. Texas Agricultural Experiment Station. 4-5.

Taylor, C. A. Jr., N. E. Garza. Jr., J. E. Huston, R. A. Moen, and T. D. Willingham. 1998b. Pre-breeding nutrition effects on ovulation rate in angora nannies. Sheep and goat, wool and mohair. Texas Agricultural Experiment Station. 3-4.

Tripp, M. W., J. C. Ju, T. A. Hoagland, J. W. Riesen, X. Yang, and S. A. Zinn. 1999. Influence of somatotropin and nutrition on bovine oocyte retrieval and in vitro development. Theriogenology. 53:1581-1590.

Wiltbank, J. N., E. J. Warwick, E. H. Vernon, and B. M. Priode. 1965. Factors affecting net calf crop in beef cattle. J. Anim. Sci. 20:409 abst.

Yaakub, H., D. O'Callaghan, and M. P. Boland. 1998. Effect of type and quantity of concentrates on superovulation and embryo yield in beef heifers. Theriogenology. 51:1259-1266.