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The Influence of Fluorescent Light on the Development of *in vitro* Fertilized Bovine Oocytes

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INTRODUCTION

Advances in *in vitro* fertilization and *in vitro* culture techniques have allowed considerable progress in identifying physiological requirements of mammalian embryos. Parrish et al. (11) reported a major breakthrough on *in vitro* fertilization when his group identified heparin as an important factor for the capacitation of spermatozoa. Capacitation is necessary for fertilization of matured oocytes. During the precoculture era of embryos (prior to 1980), the development of early preimplantation embryos was vary limited regardless of medium, medium supplement, gas atmosphere, osmolarity or pH used (7). Coculture techniques of fertilized oocytes using somatic cells during *in vitro* production, particularly of bovine embryos, has resulted in a tremendous resurgence of embryo culture research (1,2,4-6,8,9,13,15-17, 19). The most often used coculture system is oviductal epithelium (4,9,19), and it is also the most efficient system compared to others (16). Within recent years efforts by many have been undertaken to develop defined medium for oocytes/embryos. Although progress has been made in this area with laboratory animals, defined media for livestock species have thus far proven inadequate for supporting normal development of fertilized eggs to the blastocyst stage of embryonic development (3,12)

In vitro fertilization and *in vitro* culture of embryos is usually performed in laboratories under the presence of fluorescent lights. Oocytes/embryos may be exposed to fluorescent lights ranging from a few minutes to as long as 1/2 hour. Light in the range of fluorescent illumination causes the formation of free radicals in medium which is thought to have deleterious effects on embryo development (18). Although many researchers consider fluorescent lighting to be detrimental to embryo development, there are no documented studies to demonstrate a causal effect in livestock (20). Therefore, this study was designed to identify the effects of fluorescent light on the early stage bovine embryo under conditions that are commonly used in *in vitro* fertilization laboratories.

MATERIALS AND METHODS

The protocols for *in vitro* matured oocytes (IVM), *in vitro* fertilized oocytes (IVF) and *in vitro* cultured embryos (IVC) were based on Parrish et al. (10), Sirard et al. (16), Bavister et al. (2), Thibodeux et al. (17), and Pinyopummintr et al. (12) with modification according to the experimental design as follows.

Collection of oocytes. Bovine ovaries were obtained from the slaughter-house of E. A. Miller, Hyrum, Utah, kept in saline containing antibiotics and transported to the Physiology Lab., Utah State University, Logan, Utah. Follicles were aspirated using an 18-gauge needle and a vacuum pump. Only oocytes with an intact cumulus complex and evenly granulated plasma were used. Oocytes were washed one time in Hepes-TALP (16) and three times in maturation medium.

In vitro maturation. Oocytes were matured in M-199 (Gibco) with supplemented 10% (non-heat treated) fetal bovine serum (FBS, HyQ), 0.25 mM pyruvate-Na, 5 μg/ml LH (Sigma), 0.5 μg/ml FSH (Sigma), 1 μg/ml 17B-estradiol (Sigma) and 50 μg/ml gentamicin. Cumulus oocyte complexes were cultured at 39 °C, 100% humidity and 5% CO_2 for approximately 24 hours until complexes were fully expanded. Matured oocytes were transferred into into fert-TALP which contains 10 μg/ml heparin.

Preparation of oviduct epithelium for coculture. Epithelium cells were recovered by flushing the oviduct with Dulbeccos phosphate buffered solution (D-PBS). Cells were washed three times in M-199 plus 10% FBS and then cultured in M-199 plus 10% FBS at 39 °C, 100% humidity, 5% CO_2 for two days.

In vitro fertilization. Liquid nitrogen frozen bovine sperm were thawed at 37 °C for one minute and maintained at room temperature (approximately 32 °C). Live sperm were separated by a 45% and 90% Percoll gradient in modified sperm-TALP and maintained at 39 °C until ready for use. Modified sperm TALP has no pyruvate, pen/str or BSA. Live sperm were washed twice with complete sperm-TALP. Percoll separated sperm were added into fert-TALP in a final concentration of 4 million sperm per ml of medium. The egg-sperm mixture was placed in a 39 °C, 5% CO_2 and 100% humidity incubator for IVF culture.

In vitro culture. After in vitro fertilization culture of approximately 17-22 hours, oocytes were denuded of cumulus cells by vortexing 3 min. The denuded oocytes for all treatments were placed into co-culture with fresh oviductal epithelial cell vesicles with M-199 in a humidified 5% CO_2 atmosphere at 39 °C. Treatments consisted of: 1, control inside the incubator with no exposure to fluorescent light; 2, control outside incubator with no exposure to fluorescent light; 3, exposure to fluorescent light for 15, 30 and 45 minutes; and 4, fluorescent light exposure through a yellow plastic membrane designed to inhibit the effects of fluorescent light for 15, 30 and 45

minutes. Approximately two replicates with 50 oocytes each were used for each treatment group. Light exposure treatments was with a Westinghouse F48T12/CW/HO cool white fluorescent light at a distance of 30 inches. Cleavage rate (embryos developing to the 2-cell stage) was identified for each treatment group at approximately 45 hours after first exposure to sperm. Embryo development to the morula, blastocyst, expanded blastocyst and hatched blastocyst was evaluated at day 9 of coculture. Student t-test was used to identify significant differences.

RESULTS AND DISCUSSION

The effects of fluorescent light on the early preimplanted bovine embryo is shown in table 1. Approximately 100 oocytes (95 - 133) were used for each treatment group. There were no significant differences between repeats within each treatment group (control, yellow filter and non yellow filter groups) and therefore the percent cleavage rate was pooled. For the control the pooled clevage rate is 78.87. For the yellow filter and non filter groups it was 79.51 and 72.16, respectively. There was a significant difference ($P \leq .05$) between the light exposed and light filtered groups. One of the replicates in the 45 minute light exposure group (without filter) had an unusally high cleavage rate (86.35) verses 62.69 in the other replicate. Since this was tolally unexpected and unexplainable, we evaluated the control verses noncovered light exposure groups dropping out the 86.35 cleavage rate data. The cleavage rate was 69.08 after dropping out this data. Under this condition, there was a significant difference ($P \leq .05$) between the controls and the oocystes exposed to fluorescent light. There was no significant difference between the control and light filtered group.

There was no difference in cleavage rate to the 2-cell stage of development with increased time to fluorescent light, which suggests that whatever factors that cause the reduction in cleavage rate are in full effect within the first 15 minutes of exposure.

Once embryos develop to the 2-cell stage there does not appear to be any lingering effect of fluorescent light on subsequent embryo development (table 1). Upon pooling the number of embryos that developed to the morula and blastocyst stage of embryonic development, the inside and outside control was 45.96 and 40.51, respectively. The percent of embryos that developed to the morula and blastocysts stages of development in the yellow covered treatment was 58.10, 47.78 and 51.45, respectively for 15, 30 and 45 minutes exposure to fluorescent light. For direct light exposure group the percentage that developed to morula and blastocysts was 47.37, 47.83 and 43.43, respectively for 15, 30 and 45 minutes of exposure. Although the highest rate of development occurred in the treatment with the yellow filter, the difference was not significant at

the $P \leq .05$ level when compared to controls or the direct light exposure group.

Figures 1-6 illustrates the different stages of oocyte/embryo development as observed in this study.

The major concern of exposing embryos to fluorescent light is that the light waves emitted in the fluorescent range causes an increase in free radicals (18). This is a particular concern under the conditions that oocytes are handled during in vitro prodfuction of embryos. The production of free radicals is quite high under the atmospheric oxygen tension during *in vitro* production of embryos (10).

A free radical is an atom or a group of atoms with an unpaired electron valence. Free radicals are often produced by photolysis or pyrolysis in which a bond is formed without forming ions. Because of the free valency, free radicals are often extremely reactive to cell membranes, cellular products and DNA. They are also reactive to components in culture media that may be essential for normal cell growth and metabolism. It has been reported that yellow filters block specific fluorescent light emissions in the range that enhances free radical formation. The efficacy of using a yellow filter is born out in this study since the higher cleavage rates occurred under a yellow filter and although not statistically significant, embryo development to the morula and blastocyst stages of embryonic development was higher than direct oocyte/embryo exposure to fluorescent light.

Based on the results of this study we would recommend that IVF laboratories working under fluorescent lights take precautionary measure to reduce the exposure of embryos to light or to use yellow filters.

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Table 1. *In vitro* development of bovine embryos after exposure to 15, 30 and 45 minutes of fluorescent light.

Exposure (Min.)	No. of oocytes	Stage of development (%) 2 days (2-cell)	9 days Morula	9 days Blastocyst(B)	9 days Exp. B	9 days Hatched B
Control (inside)[a]	95	77.90	25.68	12.16	6.76	1.35
Control (outside)[b]	99	79.80	18.99	11.39	8.86	1.27
Yellow cover - 15	101	73.26	32.43	14.86	8.11	2.70
- 30	102	81.37	24.10	13.25	6.02	2.41
- 45	124	83.06	26.21	13.59	9.71	1.94
No cover - 15	109	69.73	31.58	11.84	3.95	0
- 30	128	71.88	26.09	11.96	9.78	0
- 45	133	74.44	21.21	12.12	9.09	1.01

[a]Ooctyes were placed in an incubator and not exposed to light.

[b]Oocytes were maintained alongside yellow filter membrane cover and no cover treatments but not exposed to light.

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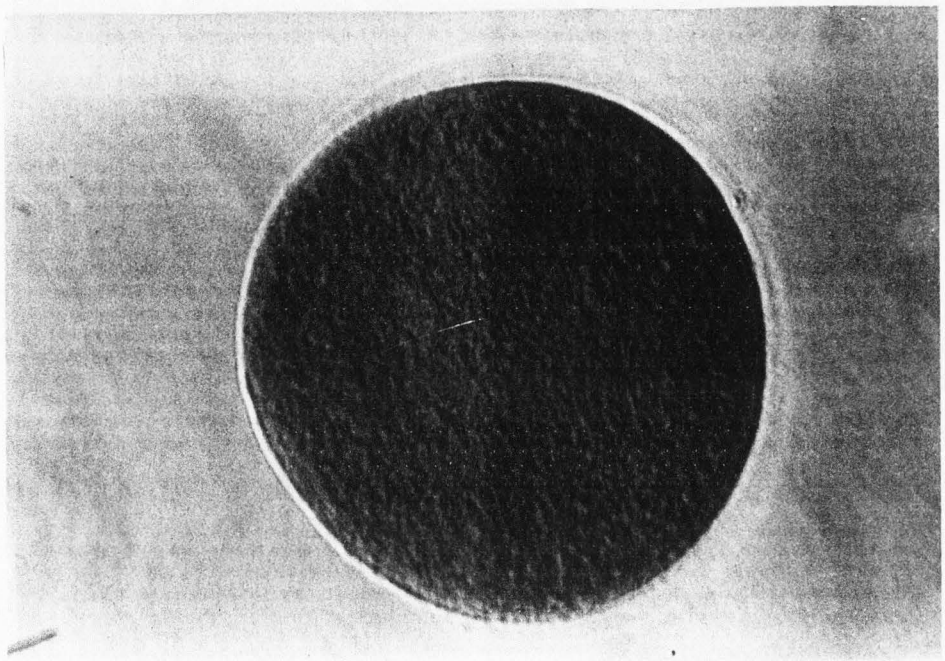

Figure 1. *In vitro* matured bovine oocyte.

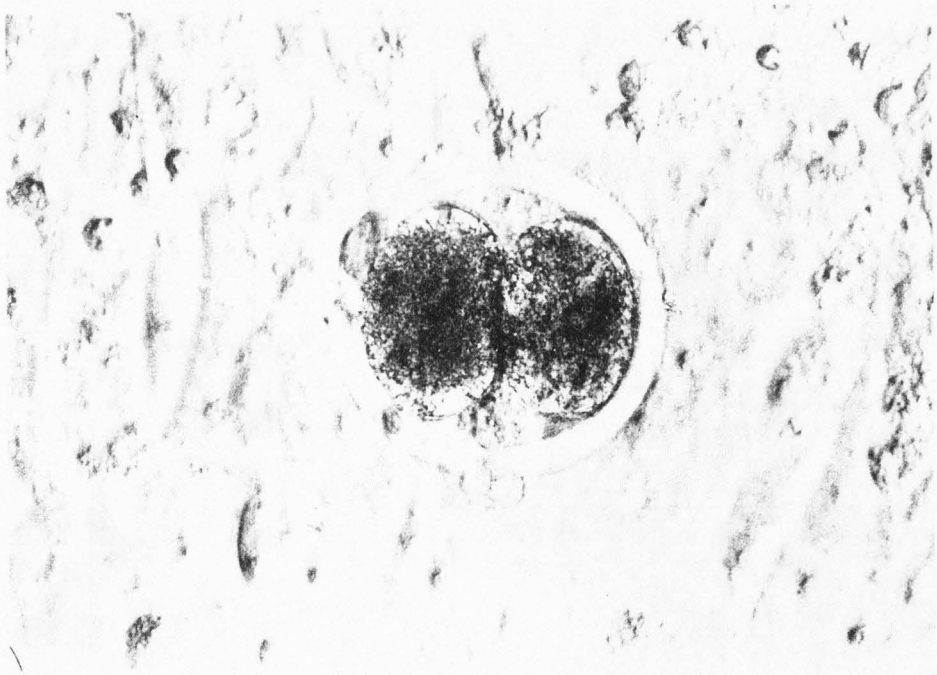

Figure 2. Two-cell bovine embryo

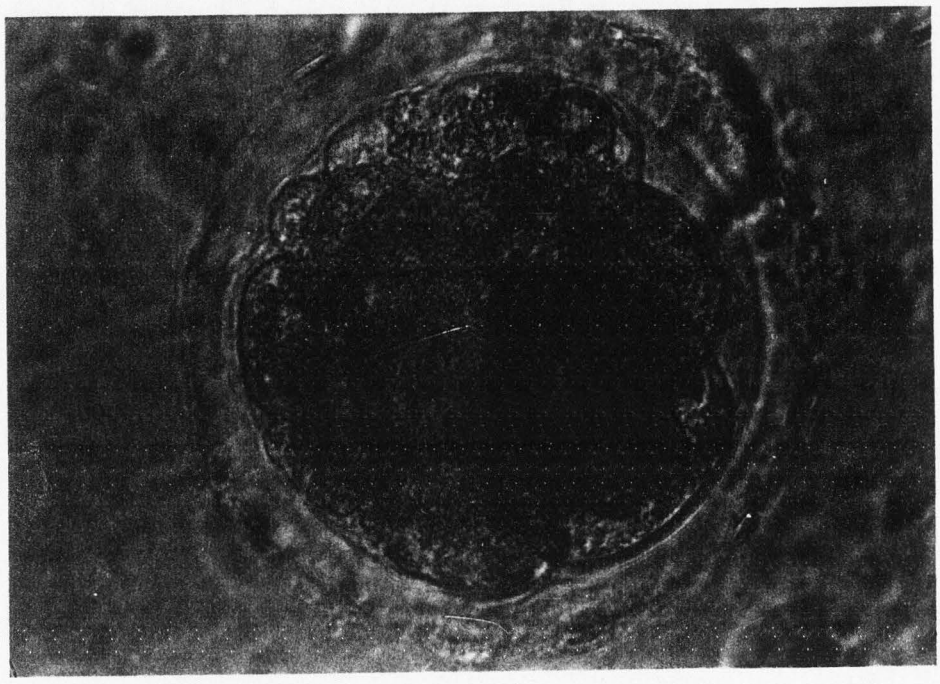

Figure 3. Bovine morula

Figure 4. Bovine blastocyst

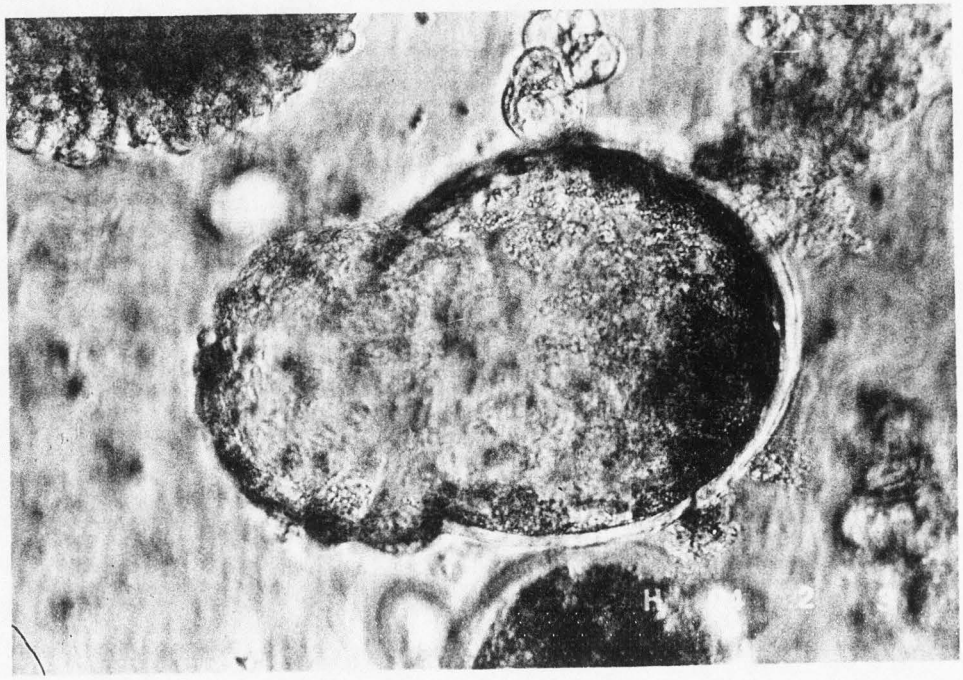

Figure 5. Bovine hatching blastocyst

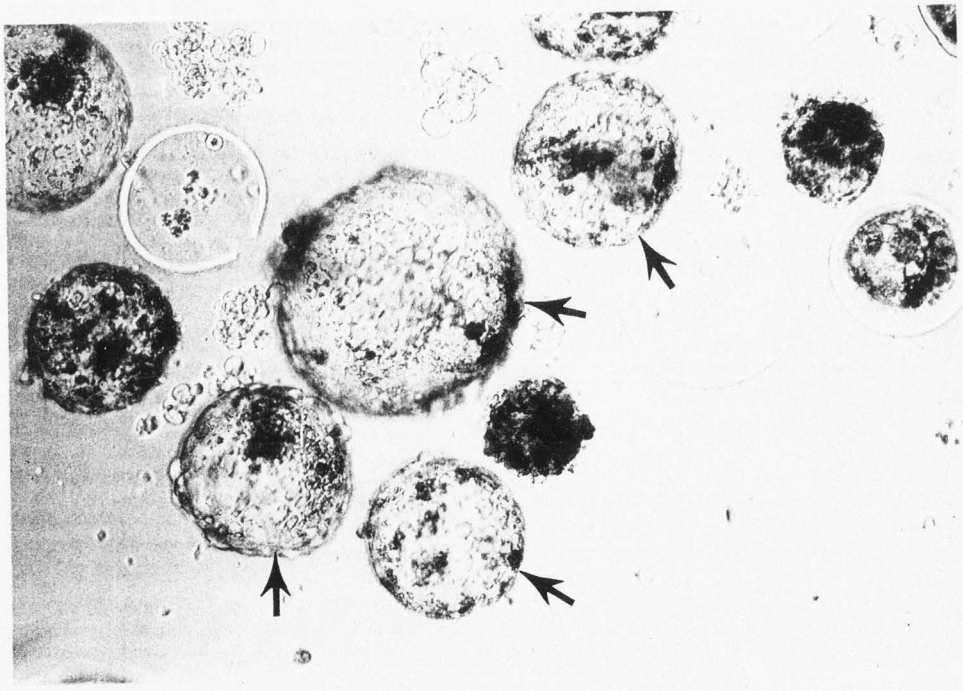

Figure 6. Bovine hatched blastocyst (arrow)