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Low Laboratory Relative Humidities Appear to Impact Initial Conception Rates but Not On-Going Pregnancies in an ART Program

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ABSTRACT

It is also well established that well-controlled humidity is essential to Assisted Reproductive Technologies (ART) culture conditions and necessary for optimal embryo development. However, to what extent fluctuating lab Relative Humidity (RH), especially low RH, might impact the culture environment is unclear. Changing weather patterns have led to more extreme periods of extended low humidities across areas of the Southwestern U.S. This, in turn, has led to lower building RH at this facility and provided an opportunity to examine the role of lab RH on culture conditions because of a concern that lower building humidity might be impacting success rates a quality management review of data from a Southwestern U.S. ART program comparing laboratory RH to embryo development and pregnancy outcomes. Data were divided between high and low room RH periods and analyzed using t-test comparisons. A total of 79 cycles were reviewed. Preliminary observations demonstrated that the majority of the chemical pregnancies (69.4%), occurred during cycles conducted during periods of relatively high laboratory RH. However, once established, equal percentages of pregnancies progressed to heartbeat (63.6% vs. 69.9%; low RH: high RH, respectively). Comparisons were also made for overall embryo development and embryo quality during the period. Results suggest that laboratory RH might impact the establishment of pregnancy in ART patients but have little effect once pregnancy is established. Low lab RH might result in shifts in media osmolarity or shifts in gas control and pH, adversely affecting embryo development. Ongoing studies are examining this relationship.

Introduction

It is commonly understood that embryo growth and quality in an Assisted Reproductive Technologies (ART) program are directly dependent on the culture environment in which embryos are grown. Therefore, maintaining a stable laboratory environment is essential to program success. Several studies have shown that environmental contaminants (organic materials, construction dust, ...) can significantly negatively impact culture and, therefore, pregnancy outcomes [1-4]. Further, it is also well established that humidity in the culture environment plays an important role in maintaining stable culture conditions [5]. However, what is less understood is the impact local seasonal weather patterns might be having on laboratory stability. There can be little debate that weather patterns have become more extreme over the last few decades [6,7]. In the Southwestern United States, such shifts have led to prolonged periods of drought and extremely low humidity. Even with building environmental controls, our program experienced numerous periods where laboratory humidities were below 15%. Over the same time period we saw significant fluctuations in pregnancy rates. Therefore, the laboratory undertook a quality management review to determine if any relationship existed between the fluctuating room humidities, pregnancy rates, and other factors associated with the ART program. What follows is a report of our observations.

Materials and Methods

Before conducting a full-scale program review, a small preliminary quality control exercise was conducted to determine if the low laboratory humidities could affect culture conditions due to exposure of media to ambient environments. In theory, the small volumes we work with could undergo significant shifts in osmolarity due to the desiccation of media during periods when the media (and embryos) had to be out in the open laboratory environment (such as ICSI procedures). Organ culture dishes were outfitted with electrical leads to allow measurement of resistance in culture media. A change in resistance would be a direct measurement of increased osmolarity due to sample dehydration. One-half milliliter of embryo culture media was added to the central organ well (a volume ten times larger than most culture conditions, which in theory should take longer for a detectable shift in resistance representing significant evaporation), and the electrical resistance was measured every minute. Shifts greater than 10% in electric conductivity were considered significant and at a level where media had suffered significant evaporation. The experiments were conducted both with and without an oil layer. As will be discussed in detail in the results below, shifting resistance patterns were seen in less than 5 minutes when media were left unprotected on the lab countertop. This time period was only extended to 30 minutes in samples overlaid with oil.

Given these preliminary findings, it appeared reasonable to assume that shifts in laboratory humidities might affect outcomes, and a data review was conducted comparing laboratory humidity to cycle outcomes. Cases were reviewed for two years from 2011-12, a period of extreme drought and extreme seasonal variation in humidity at this location. Comparisons were made between cases where relative humidity (R.H.) in the lab were < 25% on the first day of culture compared to those with R.H. over 25%. The 25% value was selected as the splitting point as it historically represented the mean R.H. for the laboratory since its establishment. All data used were obtained from laboratory records and include

1. Embryo development for all cases at Day 3,
2. blastocyst development rates for cases with blastocyst transfer (note, because of the time period of the review, Day 3 transfer was a common option),
3. Chemical pregnancies
4. On-going pregnancy with a heartbeat. A total of 79 cycles were reviewed. Outcome comparisons were made using a simple student's-T test.

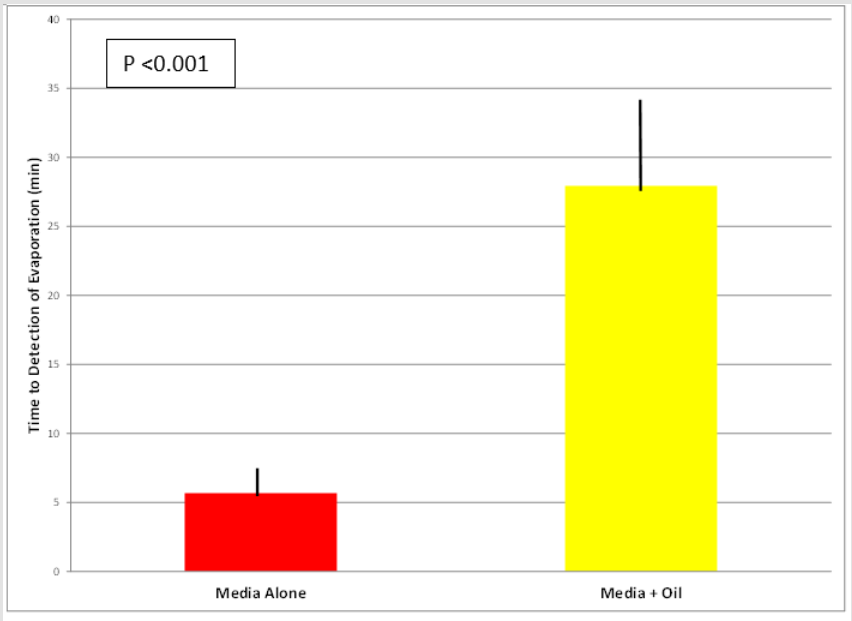

Figure 1: Evaporation rates of an Assisted Reproductive Technologies media during periods of extremely low room ambient relative humidity (~20%; mean +/- STD). Media desiccation would lead to osmotic concentration and potentially affect embryo growth rates.

Results

Preliminary Study

A literature review suggests little to no research exists on the risk of dehydration during culture. This possibly reflects the fact that culture procedures are generally conducted in high-humidity environments and, in the case of embryos, is often done under oil to prevent exposure to open air. However, embryo culture is different, as it potentially involves numerous steps outside the protective environment of an incubator. Media was placed on the countertop to assess the real

risk of dehydration damage to determine how fast evaporation would transpire. Using a simple Ohm Meter, changes in resistance were measured over time. On the day of the experiment, the ambient lab R.H. varied between 19-21%, and the temperature was 21oC. The experiments were repeated 5X for samples exposed to the open air and those overlaid with oil. In this study, samples with a volume of 0.5 mL suffered significant desiccation in as little as 5 min when left unprotected on the lab countertop during periods of low humidity (Figure 1). The addition of an oil overlayer extended the time to detectible changes to just under 30 mins but still suggested the low ambient R.H. of the culture facility might be influencing embryo culture conditions, embryo development, and potentially pregnancy outcome, leading to the records review.

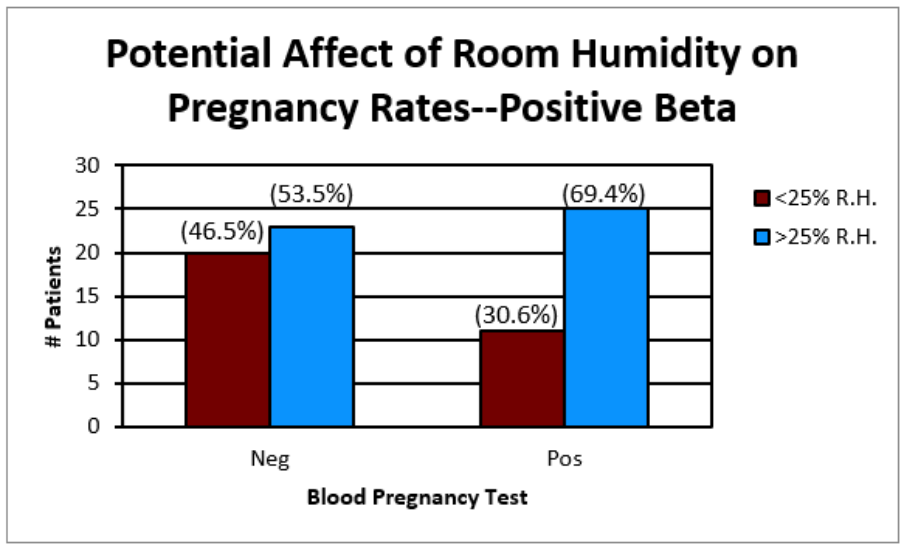

Figure 2: The establishment of pregnancy, regardless of the day of transfer in an Assisted Reproductive Technologies program, appears to be associated (P =0.10) with ambient relative humidity and suggests periods of low ambient humidity negatively impact the pregnancy rate.

Data Review

A total of 79 ART cycles were reviewed as part of this quality management exercise. Because of the time period of the study, the clinic was still performing a significant number of Day 3 versus Day 5 transfers, with 31 cases resulting in Day 3 transfers and 48 occurring on Day 5 or 6 over the two years. The region's normal weather pattern consists of two rainy periods with higher R.H. and two dryer periods with extremely low R.H. The weather patterns were dryer in the study time period, with overall R.H. in the lab during the 24 months averaging 25.1% (Range 16-42%). Data were grouped by R.H. on the day of retrieval, which obviously correlates to the first day of culture. Grouping in this manner identified 31 cases falling during periods of relatively low humidity (< 25%) and 48 during periods of relatively high humidity (>25%). A total of 36/79 cycles reviewed resulted in a positive pregnancy test (45.6%). The data suggested a trend (p = 0.10) toward higher pregnancy rates during wetter periods, with two-thirds of the detected :>0L (chemical pregnancies) occurring during the more humid periods (69.4 %,) (Figure 2). Further, if the data is further broken down by day of transfer, there was no difference between Day3 and Day 5/6 transfers at either R.H. level (P = 0.68), but the trend toward increased pregnancy during periods of high humidity was maintained (P =0. 09) (Figure 3).

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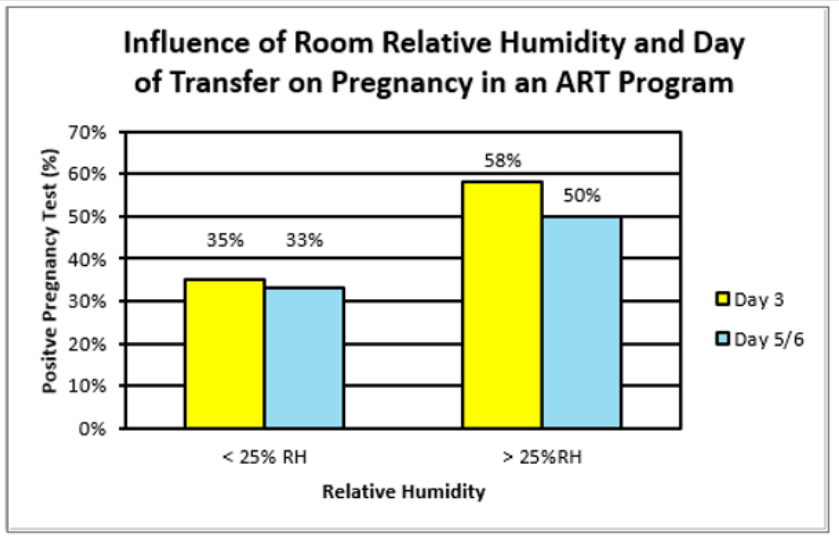

Figure 3: ART cycles resulting in positive pregnancy tests based on the day of transfer and the relative humidity (R.H.) during the culture period. Data suggest a trend toward lower pregnancy rates from embryos cultured during periods of low R.H. (P = 0.09).

Further, cumulative embryo development was equal in all groups on the third day of development (P = 0.300) (Figure 4). However, in the 48 cases with Day 5/6 transfers, a higher percentage of embryos reached the blastocyst stage of development during periods of high lab R.H. (P <0.030 (Figure 5). Collectively, these data would appear to suggest that lower humidities in the lab might adversely affect cycle outcome and potentially affect the growth potential of some embryos, especially in extended culture where embryos would be potentially exposed to more fluctuations in the culture environment. However, the data also suggest that the growth potential of embryos that established a pregnancy do not appear to be adversely affected by lab R.H. during culture, as the percentage of embryos that continued on to heartbeat (P=0.781) (Figure 6) were essentially equal in both groups. Collectively, these data would appear to suggest that lower humidities in the lab might adversely affect cycle outcome and potentially affect the growth potential of some embryos, especially in extended culture where embryos would be potentially exposed to more fluctuations in the culture environment. However, the data also suggest that the growth potential of embryos that established a pregnancy do not appear to be adversely affected by lab R.H. during culture, as the percentage of embryos that continued on to heartbeat (P=0.781) (Figure 6) were essentially equal in both groups.

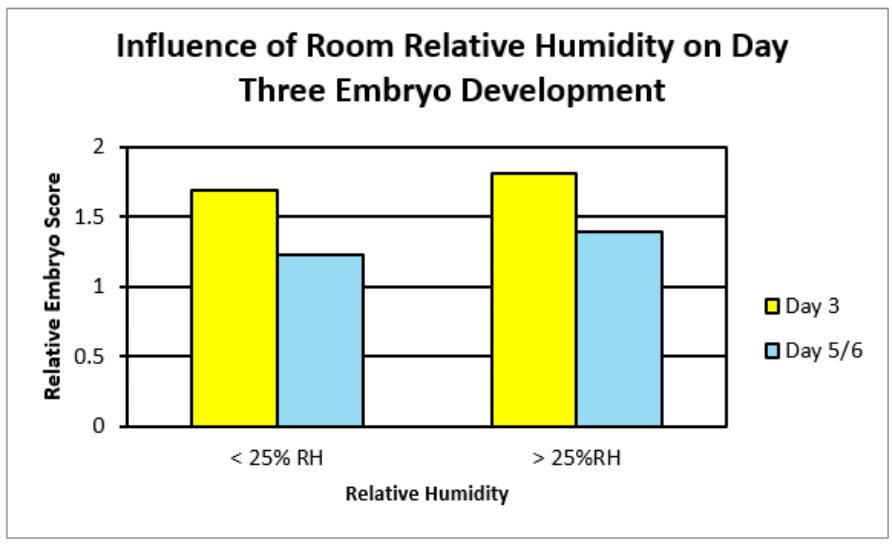

Figure 4: Relative embryo score on day three of culture suggests little influence of culture room relative humidity on early embryo development (P=0.30).

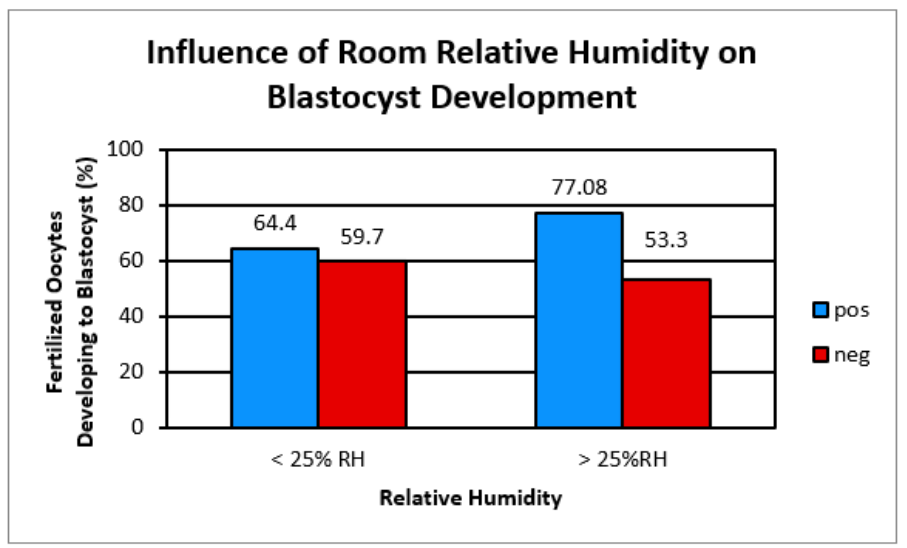

Figure 5: Rate of blastocyst development in Day 5/6 cultures as influenced by the relative humidity (R.H.) of the culture laboratory, suggesting low R.H. may slow embryo development in the latter stages of embryo culture ($P < 0.03$).

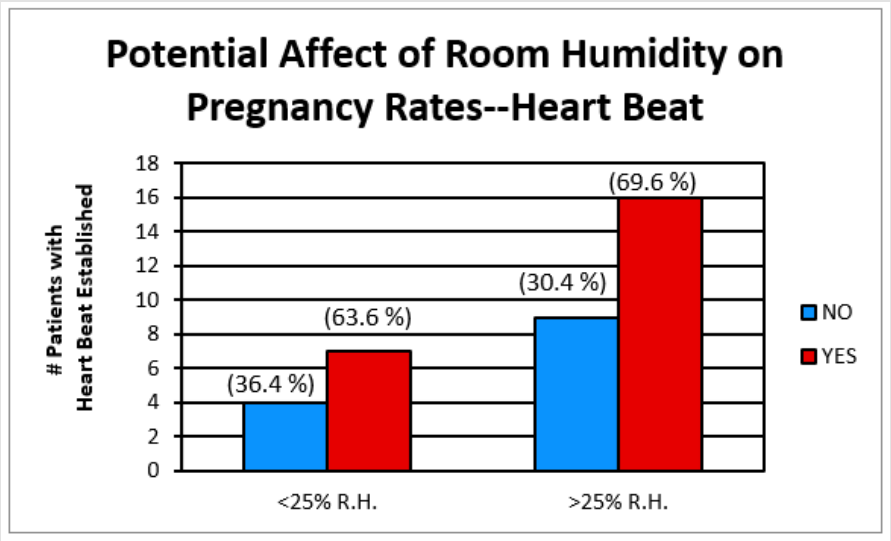

Figure 6: Once established, the rate of ongoing pregnancies reaching heartbeat from an Assisted Reproductive Technologies program appears unaffected by the laboratory relative humidity during the culture period ($P = 0.781$).

Discussion

The ART laboratory is relatively unique among cell culture facilities, as embryos can potentially be removed from the culture environment and manipulated on a daily basis. Further, due to the nature of fertilization (especially with ICSI), genetic testing, transfer, and freezing procedures, the embryos may be exposed to the general laboratory environment multiple times during the in vitro culture. Data from the present experiment suggests that small volumes of media can undergo significant dehydration in relatively short periods of time. Embryos present in such media would potentially be exposed to suboptimal conditions, not only due to media dehydration but also temperature and pH shifts [8,9] which might negatively impact the embryo and its survival. Data from the present study suggests embryo growth and development and the ability of the embryos to establish a pregnancy, which might be adversely impacted by low R.H. within the embryo laboratory. Further, the data suggest there may be a relationship between the time the embryos spend in the lab and the level of

the effect. However, embryos that survive exposure to low R.H. during the ex vivo culture period and implant all seem to have the same growth potential as embryos developed during better R.H. conditions. This would suggest that a low R.H. environment might serve to "weed out" marginal embryos by preventing their development. While great strides have been made in embryo culture over the last two decades, there is still a need to understand the optimal conditions for embryo culture [10-12].

Further, recent changes in weather patterns [13] have not only led to more extreme weather events4,5, but also affected the availability of energy sources to maintain the internal environments of work facilities, such as ART Labs [14]. Therefore, fully understanding the negative impact fluctuations in the laboratory environment (temperature, R.H., and even the power grid) will be essential in maintaining the standard established for ART over the last forty years.

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