Propofol as Intravenous General Anesthesia for Transvaginal Oocyte Retrieval in Assisted Reproductive Technique: Effect on Oocyte Quality*

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Background: Propofol, used for IV induction of anesthesia in ART, has been suspected of damaging oocytes. Its levels have been shown to increase in follicular fluid during oocyte retrieval.
Objective: To assess whether exposure to increasing concentrations of propofol has a measurable effect on the quality of oocyte for in-vitro fertilization.
Materials and Methods: A cohort of 40 women underwent IV anesthesia using propofol and fentanyl. Time of anesthesia from IV injection of propofol was measured. First and last oocytes were cultured separately. The mean time from IV injection to first follicle aspiration was 9.5 minutes.
Results and Conclusion: This study showed that exposure to the IV anesthetic drug propofol had significant effect on the grading of oocytes. The subjects with grade 1 oocytes significantly decreased at the last oocyte retrieval while grade 2 oocytes significantly increased at the last oocyte retrieval. We conclude that the time elapsed between retrieval of the first and last oocyte does affect oocyte quality.

Key words: anesthesia, propofol, oocyte quality, assisted reproductive technique

Introduction

General anesthesia with intravenous (IV) agents used to be the most popular form of pain control for transvaginal oocyte retrieval in assisted reproduction.[1] However, studies have shown that different anesthetic agents have different effects on oocyte fertilization and embryonic development.[2-5] Vincent, et al.[3] found that propofol was associated with lower clinical and ongoing pregnancy rates when compared to isoflurane. But other studies revealed no detrimental effects or negative outcomes,.[4,5] The concentrations of some anesthetic agents have been measured in the aspirated follicular fluid,[6-8] the latest of which was propofol.[5] Direct observations in animal models also tested the possible toxic effect on oocyte fertilization and early embryonic development.[9,10] Depypere, et al.[11] and Jansseswillen, et al.[10] reported a dose- and time-dependent detrimental effect of propofol on the fecundability of mouse oocytes. These findings have been confirmed in two recent papers, where it was also shown that maturation rate was decreased by high propofol concentration[9] and cleavage to blastocyst stage was inhibited.[12] On the other hand, several authors found no detrimental effects or negative outcome in human or animal in vitro fertilization when propofol was used.[4,6] Sia-Kho, et al.[13], even suggested that propofol might be beneficial for the cleavage of oocytes and fertilization rate. Retrospective studies by Borsatti, et al.[14] and Rosenblatt, et al.[15] reported no difference in

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implantation rate or pregnancy rate when propofol was compared with other anesthetic techniques for oocyte retrieval.

Propofol is chemically unrelated to earlier anesthetic drugs. This highly lipophilic agent has a fast onset and short, predictable duration of action due to its rapid penetration of the blood-brain barrier and distribution to the central nervous system.[16] It is a very popular anesthetic of choice for various ambulatory procedures including oocyte retrieval.[8,17,18] Recently, a study showed that when total IV anesthesia is maintained with continuous propofol pump, a gradual, time-dependent, linear increase of its concentrations is observed in follicular fluid.[19] Propofol is a 2,6-diisopropylphenol which is a short-acting IV anesthetic agent suitable for induction and maintenance of anesthesia. It has a rapid onset of action ~30 s following an IV injection. The recovery from anesthesia is also rapid. The mechanism of action of Propofol, like all general anesthesia, is poorly understood. It has been reported that Propofol inhibits polysynaptic excitatory neurotransmission in the olfactory cortex and spinal cord which is thought to be mediated by the N-methyl-D-aspartate receptor channel, in addition to the potentiation of y-aminobutyric acid-mediated synaptic inhibition in a variety of systems.[20]

This study evaluated the effect of propofol on oocyte quality or grading during general anesthesia. Coetsier, et al.[8] demonstrated that propofol accumulation in follicular fluid is time dependent. Their conclusion was based on a pharmacokinetic profile using venous blood samples. However, animal studies have shown that arterial and venous sampling may lead to a considerable difference in drug concentration.[21,22] and that arterial sampling may be more accurate for the evaluation of distribution, elimination and pharmacodynamics effects.[23] Since the accumulation of propofol in follicular fluid might have effects on in vitro fertilization and early embryo development and thus might have important clinical and ethical complications, it would be important to correlate duration of propofol exposure during transvaginal oocyte retrieval to oocyte grading, since this information may possibly restrict the amount of propofol ultimately administered during anesthesia. In an attempt to document possible differences in fertilization rates and early embryo development potentially attributable to propofol, we separated first aspirated oocytes from last aspirated oocytes during propofol/fentanyl-induced anesthesia for oocyte retrieval.

Objectives

1. To evaluate the quality of oocytes, as to the *polar body* and *cytoplasm*, of women undergoing transvaginal oocyte retrieval for assisted reproductive technique given propofol as intravenous general anesthesia.
2. To compare the quality or grading between the first aspirated oocytes and last-aspirated oocytes retrieved after transvaginal oocyte retrieval.

Materials and Methods

The study group included 40 unpremedicated women scheduled for oocyte retrieval. The general anesthesia protocol consisted of IV fentanyl 0.5-1.0 mg followed after 2 minutes by IV propofol 1.5-2.0 mg/kg. An adequate level of anesthesia was maintained by repeated boluses of propofol as clinically appropriate. The time elapsed from the initial administration of propofol to the aspiration of the last follicle was recorded.

Oocyte retrieval was performed transvaginally with ultrasound guidance. The retrieval of immature oocytes is very similar to the retrieval of mature oocytes from stimulated follicles. The vaginal vault was cleansed with antibacterial soap and sterile water. The sterile biopsy guide and a 5 mHz transvaginal probe was inserted into the lateral fornix. An ultrasound evaluation was performed and all follicles identified as 6-12 mm were punctured using 800-100mm Hg pressure until all follicular contents including fluid and cells were curetted from the follicle. The fluid was collected with flushing the follicle into a test tube with 3ml of culture media maintained at 37°C. All patients undergoing oocyte retrieval received IV sedation with Fentanyl and Propofol. All of the

follicles sized >/= 6mm were aspirated. The needle was a 17 gauge, 30cm long Casmed double lumen aspiration with a short bevel.

When the follicular fluid was obtained, it was filtered by gravity and rinsed with G-MOPS™ Handling media (Vitrolife) to remove any red blood cells. The fluid remaining after filtration was decanted into petri dishes which were scanned under a stereomicroscope for the identification of immature oocytes. These immature oocytes were graded and placed into Nunc dishes with G-MOPS™ Handling media (Vitrolife).

In 40 patients predicted to have at least 15 oocytes, the oocytes were divided and kept in groups according to the sequence of aspiration. Thus, about a third of oocytes were designated 'early', a third were designated 'last' and all the rest were call 'intermediate'. Special care was taken to ascertain that the last aspirated follicle would be approximately the same size as the first. The oocytes were graded according to polar body and its cytoplasm.

Polar body classification:

Grade 1 - round or ovoid. Intact and with smooth surface; Grade 2 - round or ovoid. Intact but with a rough surface; Grade 3 - Fragmented; Grade 4 - Large, mostly associated with a large perivitelline space.

Cytoplasm:

Grade 1 - translucent yellowish colored with homogenous and fine granularity of the ooplasm; Grade 2 - an excessive and dark granularity affecting the whole cytoplasm maybe associated with degenerative oocyte; Grade 3 - centrally located granular cytoplasm, appearing as a larger, darker, spongy granular area; Grade 4 - granularity, light, heavy.

Results

Forty patients who were candidates for ART underwent the oocyte retrieval process. Their mean age was 34.13 years, range 25-41 years. Their diagnoses included tubal disease in 5 (12.5%) subjects, endometriosis in 4 (10%), anovulation 14 (35.0%), male factor 8 (20.0%) and unexplained 9 (22.5%). The mean years of infertility was 4.44 years ranging from 1 to 19 years where >80% were infertile for 1-5 years.

Table 1. Demographic characteristics of subjects.

	Frequency (n=40)	Percentage
Age		
25 - 30	9	22.5
31 - 35	11	27.5
36 - 40	18	45.0
41 - 45	2	5.0
Mean ± SD = 34.13 ± 4.03		
Indication		
Anovulation	14	35.0
Endometriosis	4	10.0
Male Factor	8	20.0
Tubal disease	5	12.5
Unexplained	9	22.5
Years of Infertility		
1 - 5	33	82.5
6 - 10	5	12.5
11 - 15	1	2.5
16 - 20	1	2.5
Mean ± SD = 4.44 ± 3.55		

Oocytes were retrieved from all patients who underwent immature oocyte retrieval. The retrieval time for the 1st oocyte retrieval ranged from 1 minute to 27 minutes with a mean of 9.58 minutes. Time of retrieval for the last oocyte ranged from 10 minutes to 82 minutes with a mean of 36.50 minutes.

Table 3 shows the 1st and last oocyte retrieval from time of induction of anesthesia. At 1st oocyte retrieval, 33 (82.5%) were grade 1 and 7 (17.5%) were grade 2. At last oocyte retrieval, 25 (62.5%) were grade 1 and 15 (37.5%) were grade 2. Comparing the proportion of subjects with grade 1 and grade 2 oocytes, there was a significant difference noted as proven by the p value of 0.05.

The proportion of subjects with grade 1 oocytes significantly decreased at the last oocyte retrieval while grade 2 oocytes significantly increased at the last oocyte retrieval.

Table 2. Duration of first and last oocyte retrieval from time of induction of anesthesia.

	Frequency (n=40)	Percentage
Duration of 1st oocyte retrieval (in minutes)		
1 - 5	14	35.0
6 - 10	14	35.0
11 - 15	4	10.0
16 - 20	6	15.0
21 - 25	1	2.5
26 - 30	1	2.5
Mean ± SD = 9.58 ± 6.30 mins		
Duration of Last oocyte retrieval (in minutes)		
10 - 20	5	12.5
21 - 30	10	25.0
31 - 40	13	32.5
41 - 50	6	15.0
51 - 60	4	10.0
61 - 70	1	2.5
>70	1	2.5
Mean ± SD = 36.50 ± 14.36 minutes		

Table 4 shows the comparison of the different variables according to the grade 1st oocyte retrieval. At 1st oocyte retrieval, 33 (82.5%) were grade 1 and 7 (17.5%) were grade 2. Comparing the different variables between grade 1 and grade 2 oocytes, there was no significant difference noted as proven by all p values >0.05. This means that all variables listed in the table do not affect the grade of the 1st oocyte.

Discussion

In this study, the authors examined the possible effect of exposure to the IV anesthetic drug propofol

Table 3. Grading of first and Last oocyte retrieval from time of induction of anesthesia.

	Grade Last Oocyte Retrieval 1	2	Total
Grade 1st oocyte retrieval			
1	22 (66.7%)	11 (33.3%)	33
2	3 (42.9%)	4 (57.1%)	7
Total	25	15	40

p=0.05 significant

Table 4. Comparison of the different variables according to the grade of last oocyte retrieval.

	Grade Last Oocyte Retrieval 1 (n=25)	2 (n=15)	p-value*
Age			
Mean ± SD	34.40 ± 3.73	33.67 ± 4.59	0.58 (NS)
Indication			
Anovulation	8 (32.0%)	6 (40.0%)	
Endometriosis	2 (8.0%)	2 (13.3%)	
Male Factor	3 (12.0%)	5 (33.3%)	0.17 (NS)
Tubal disease	5 (20.0%)	0	
Unexplained	7 (28.0%)	2 (13.3%)	
Years of Infertility			
Mean ± SD	4.26 ± 3.95	4.73 ± 2.89	0.69 (NS)
Duration of 1st oocyte retrieval (in minutes)			
Mean ± SD	9.16 ± 5.88	10.27 ± 7.11	0.60 (NS)
Duration of Last oocyte retrieval (in minutes)			
Mean ± SD	34.84 ± 15.30	39.27 ± 12.67	0.35 (NS)

* p>0.05- Not significant; p ≤0.05-Significant

on the results of grading of oocytes. They found that exposure to the IV anesthetic drug propofol had significant effect on the grading of oocytes as shown in table 3. The proportion of subjects with grade 1 oocytes significantly decreased at the last oocyte retrieval while grade 2 oocytes significantly increased at the last oocyte retrieval. This maybe the reason why although propofol is frequently preferred, its use during transvaginal oocyte retrieval is currently being debated.[19] An animal study reported that the use of propofol had a detrimental effect on oocyte fertilization and early embryonic development.[10] However, Imoedemhe, et al.[4] reported no detrimental effects or negative outcomes in human in vitro fertilization when propofol was used. Furthermore, Rosenblatt, et al.[15] had no evidence from their data that the administration of propofol during the oocyte retrieval for oocyte donation had a negative impact on the oocytes as measured by the cumulative embryo scores, probability of a clinical pregnancy, or implantation rate. Christiaens, et al.[19] showed that a propofol-based anesthetic technique resulted in significant concentrations of this agent in the follicular fluid, in relation to the dose administered and to the duration of propofol administration. Ben-Shlomo, et al.[1], however, could demonstrate neither a correlation between the concentrations of propofol in follicular fluid and the duration of anesthesia nor a detrimental effect of high concentrations of follicular fluid propofol on oocyte quality. These authors reported that they never used a dose higher than 5 mg kg-1, whereas Christiaens, et al.[19] reported using doses of propofol up to 10 mg kg-1. Alsalili, et al.[9] evaluated the effects of different propofol concentrations (from 0.1 to 10 μg mL-1) on the ability of mouse oocytes to mature in vitro. A significant reduction in maturation rates was observed in oocytes exposed to concentrations of 10 μg mL-1 of propofol for 30 min. However, even when exposed to the highest propofol concentrations, mature oocytes had similar fertilization and cleavage rates when compared to the controls. Although propofol concentrations in the follicular fluid are directly related to the amount administered and to the duration of administration, it does not seem to have any significant adverse effect on oocyte quality or pregnancy rates.[28] When this agent is used for the induction of anesthesia only, the likelihood of a negative effect on the subsequent implantation rate may be greatly reduced, as the accumulation of propofol in the follicular fluid will be less pronounced following a single bolus administration.[5]

The shift in popularity of oocyte retrieval technique to the exclusive use of the transvaginal route has significantly shortened the duration of the procedure as well as the degree of pain involved. Nevertheless, concerns still exist regarding possible detrimental effects of the anesthetic agents on the quality of oocytes and the corresponding embryos. In this regard, there are also some studies in animals which give some support to this concern.[10] A recent study[19], gave a solid demonstration of the fact that follicle-contained oocytes are exposed to increasing concentrations of propofol as anesthesia continues. Recent data also suggest that propofol may be a safe alternative for use during assisted reproduction.[5] This method has been reported to be safe and effective.[26] Currently, in the institution a fentanyl/propofol based technique involving optimal sedation has been the technique of choice for all in vitro fertilization procedures.

Other agents such as fentanyl, alfentanil and midazolam have also been used either alone or in combination with propofol for oocyte retrieval. Although they tend to accumulate in the follicular fluid during anesthesia, follicular levels are very low compared with serum levels.[27]

Conclusion

The present study showed that exposure to the IV anesthetic drug propofol had significant effect on the grading of oocytes. The proportion of subjects with grade 1 oocytes significantly decreased at the last oocyte retrieval while grade 2 oocytes significantly increased at the last oocyte retrieval. The time elapsed between retrieval of the first and last oocyte does affect oocyte quality. It is advisable that the IVF procedure should be kept as short as possible in order to limit the accumulation of the anesthetic in the follicular fluids.

Recommendation

In planning future research, the authors recommend to measure the concentrations of propofol in the follicular fluid of oocytes as well as arterial blood samples to determine propofol concentrations. They also recommend a greater sample size to increase the accuracy of the population estimates and increase generalizability of the result. In addition, future trials should include effects of propofol on early embryo quality.

Disclosure

The authors have no financial conflicts of interest.

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